The National Toxicology Program

ANNUAL PLAN

Fiscal Year 2002

U.S. Department of Health and Human Services
Public Health Service

NATIONAL TOXICOLOGY PROGRAM

ANNUAL PLAN

FOR

FISCAL YEAR 2002

National Institute of Environmental Health Sciences/National Institutes of Health
National Center for Toxicological Research/Food and Drug Administration
National Institute for Occupational Safety and Health/Centers for Disease Control and Prevention

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PREFACE

The National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH), the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA) form the core agencies comprising the National Toxicology Program (NTP). The NTP is headquartered at the NIEHS/NIH and Dr. Kenneth Olden, Director NIEHS/NIH, also serves as the NTP Director.

The NTP is an interagency program whose mission is to evaluate agents of public health concern by developing and applying the tools of modern toxicology and molecular biology. This involves conducting toxicological evaluations of substances of public health concern, developing and validating improved (sensitive, specific, rapid) testing methods, developing approaches and generating data to strengthen the science base for risk assessment, and communicating with all stakeholders. The NTP has always drawn strength and direction from its commitment to open information exchange, adherence to impartiality, and rigorous scientific peer review. Its vision, leadership, and commitment to the concept of good science for good decisions create an atmosphere that allows flexibility in the NTP's approach toward addressing public health concerns. The NTP plays a critical role in providing needed scientific data, interpretations, and guidance concerning the appropriate uses of data to regulatory agencies and other groups involved with health-related research. Through its interactive relationship with regulatory agencies, the NTP plays an indirect, but important role in shaping public health policy.

The Program maintains an objective, science-based approach in dealing with critical issues in toxicology. The NTP conducts research and sponsors workshops through its primary agencies (NIEHS/NIH, NCTR/FDA, and NIOSH/CDC) and leverages resources through cooperative and/or collaborative agreements with other Federal agencies, academia, and industry. These interactions enhance opportunities to conduct toxicological evaluations of targeted agents, to strengthen the science base regarding mechanisms of disease etiology, and to promote the development of novel and alternative toxicology methods. Current initiatives and plans for NTP research and testing for FY 2002 are summarized herein.

The NTP welcomes comment and constructive criticism of its programs and policies. Comments may be directed to NTP Liaison and Scientific Review Office (NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709 or liaison@starbase.niehs.nih.gov).

Kenneth Olden, Ph.D.
Director

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FREQUENTLY USED ABBREVIATIONS

ATSDR	Agency for Toxic Substances and Disease Registry
CDC	Centers for Disease Control and Prevention
CERHR	Center for the Evaluation of Risks to Human Reproduction
CPSC	Consumer Product Safety Commission
DOD	Department of Defense
DPBs	Disinfection by-products
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
HHS	Department of Health and Human Services
ICCEC	Interagency Committee for Chemical Evaluation and Coordination
ICCVAM	Interagency Coordinating Committee for the Validation of Alternative Methods
NCI/NIH	National Cancer Institute of the National Institutes of Health
NCP	NTP Center for Phototoxicology
NCTR/FDA	National Center for Toxicological Research of the Food and Drug Administration
NCEH/CDC	National Center for Environmental Health of the Centers for Disease Control and Prevention
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NHANES	National Health and Nutrition Examination Survey
NIEHS/NIH	National Institute of Environmental Health Sciences of the National Institutes of Health
NIH	National Institutes of Health
NIOSH/CDC	National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention
NIST	National Instititute of Standards and Technology
NCP	NTP Center for Phototoxicology
NLM	National Library of Medicine
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
RoC	Report on Carcinogens

OVERVIEW OF THE NATIONAL TOXICOLOGY PROGRAM

MISSION AND GOALS

Today more than 80,000 chemicals are registered for use in commerce in the United States. An estimated 2,000 new ones are introduced annually to be used in products we encounter in our daily lives such as food, personal care products, prescription drugs, household cleaners, and lawn care products. The effects of many of these chemicals on human health are unknown, yet people and our environment may be exposed to them during their manufacture, distribution, use, and disposal or as pollutants in our air, water, or soil. While relatively few such chemicals are thought to pose a significant risk to human health, safeguarding the public depends upon identifying the effects of these synthetics as well as certain naturally occurring chemicals or substances and the levels of exposure at which they may become potentially hazardous to humans.

The Department of Health and Human Services (HHS) established the National Toxicology Program (NTP) in 1978 and charged the NTP with coordinating toxicological testing programs within the Public Health Service of the Department; strengthening the science base in toxicology; developing and validating improved testing methods, and providing information about potentially toxic chemicals to health regulatory and research agencies, scientific and medical communities, and the public. The NTP is an interagency program whose mission is to evaluate agents of public health concern by developing and applying the tools of modern toxicology and molecular biology. In carrying out its mission, the NTP has several goals:

- to provide evaluations of substances of public health concern,
- to develop and validate improved (sensitive, specific, rapid) testing methods,
- to develop approaches and generate data to strengthen the science base for risk assessment, and
- to communicate with all stakeholders including government, industry, academia, the environmental community, and the public.

ORGANIZATIONAL STRUCTURE AND OVERSIGHT

Three agencies, the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH), the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA), form the core for this program (Figure 1). The NTP is located administratively at the NIEHS/NIH and the Director of the NIEHS/NIH serves as the NTP Director. The National Cancer Institute of the National Institutes of Health (NCI/NIH) was a charter agency of the NTP and continues to participate on the NTP Executive Committee. The NCI/NIH carcinogenesis bioassay program was transferred to the NIEHS in July 1981. Questions and inquiries about the NTP can be directed to the NTP Office of Liaison and Scientific Review (919-541-0530 or liaision@starbase.niehs.nih.gov, see Communication and Public Outreach, page 5).

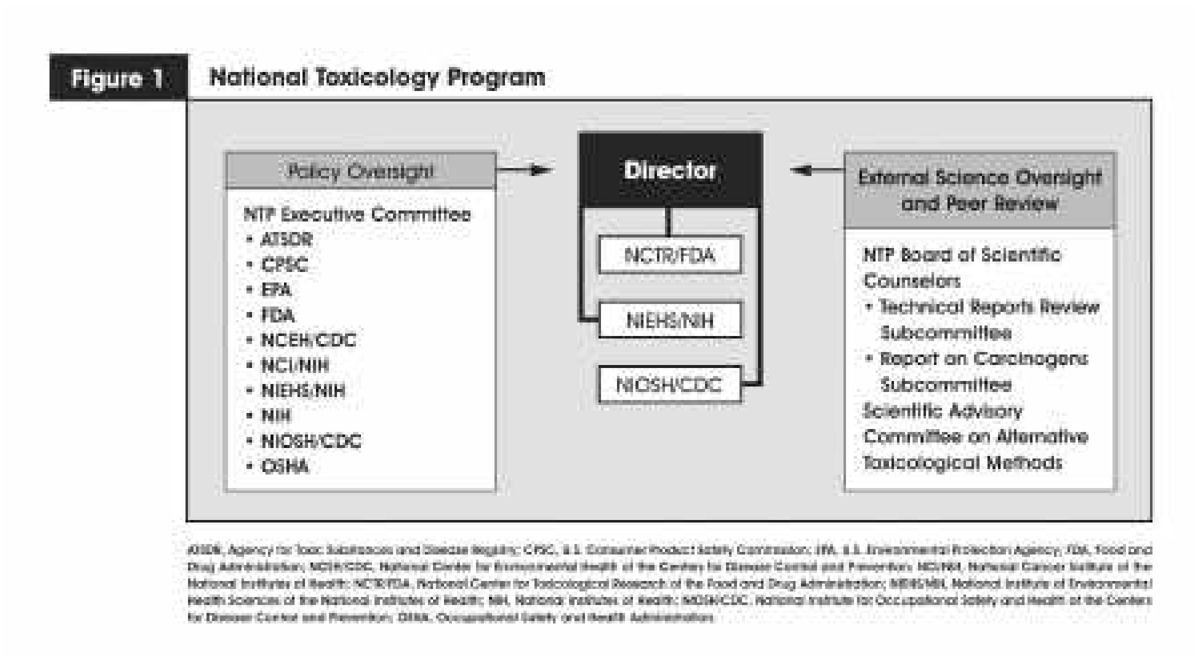
NTP Management

Dr. Kenneth Olden, Director NIEHS/NIH serves as NTP Director
Dr. Christopher J. Portier, Director, Environmental Toxicology Program, NIEHS/NIH

Agency Program Management

NCTR/FDA: Dr. William T. Allaben, Associate Director for Scientific Coordination
NIEHS/NIH: Dr. Christopher J. Portier, Director, Environmental Toxicology Program
NIOSH/CDC: Dr. Albert E. Munson, Director, Health Effects Laboratory Division

Agency staffs involved with the Program and their contact information are provided in Appendix 1.



Advisory Committees

NTP Board of Scientific Counselors

The NTP Board of Scientific Counselors ("the Board") (Figure 1), composed of up to 35 scientists primarily from the public and private sectors, provides scientific oversight to the program including its centers. A list of the current membership (as of March 2002) is provided in Appendix 2. The Board's members serve terms of up to four years. Members of the Board are distributed among the parent committee and two standing subcommittees in order to provide the necessary scientific expertise to the program. The Board's Technical Reports Review Subcommittee meets annually/semiannually and provides peer review of NTP long-term toxicology and carcinogenesis technical reports. This subcommittee also provides peer review by mail of NTP toxicity studies. The Report on Carcinogens Subcommittee of the Board provides external scientific evaluation and peer review of substances nominated for listing in or delisting (removal) from the Report on Carcinogens (see page 81). Information about the Board is available from the executive secretary, Dr. Mary S. Wolfe (NIEHS/NIH), and minutes from its meetings are accessible on the NTP web site (http://ntp-serve.niehs.nih.gov) or from Central Data Management (see page 5).

Scientific Advisory Committee on Alternative Toxicological Methods

The Scientific Advisory Committee on Alternative Toxicological Methods (SACATM) (Figure 1) was chartered in January 2002. This new, federally chartered advisory committee was established to fulfill mandates specified in the ICCVAM Authorization Act of 2000 ("the Act," Public Law 106-545). As a result, the Advisory Committee on Alternative Toxicological Methods was terminated at the end of its charter. The SACATM will provide advice to the NIEHS Director, the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and the Interagency Coordinating Committee on the Validation of Alternative Toxicological Methods (ICCVAM) on priorities and directives related to the development, validation, scientific review, and regulatory acceptance of new or revised toxicological test methods and on ways to foster partnerships and communication with interested parties (NICEATM and ICCVAM, see page 78). The committee will contain 15 members with representation as defined in the Act. Members will serve rotating terms of up to four years. The NIEHS/NIH is currently preparing a draft slate and once approved, the NTP Liaison and Scientific Review Office will move forward with setting up the first meeting; meetings will be held 2-3 times annually in public forums. Summary minutes from these meetings will be available on the NTP and NICEATM/ICCVAM web sites (http://ntp-server.niehs.nih.gov and heetp://iccvam.niehs.nih.gov, respectively).

NTP Executive Committee

The NTP Executive Committee (Figure 1) provides oversight to the NTP for policy issues. This committee is composed of the heads of Federal health and regulatory agencies (Table 1).

Table 1. NTP Executive Committee Membership Roster*

Member	Alternate	Affiliation
Henry Falk, M.D., M.P.H., Assistant Administrator	Christopher DeRosa, Ph.D. Director, Division of Toxicology	Agency for Toxic Substances and Disease Registry
Lester M. Crawford, DVM, Ph.D. Deputy Commissioner, Acting for the Commissioner	Bernard A. Schwetz, D.V.M. Senior Advisor for Science	Food and Drug Administration
Andrew C. von Eschenbach, M.D. Director	David G. Longfellow, Ph.D. Branch Chief, Chemical and Physical Carcinogens Branch	National Cancer Institute of the National Institutes of Health
Richard J. Jackson, M.D., M.P.H. Director	Thomas H. Sinks, Ph.D. Associate Director for Science	National Center for Environmental Health of the Centers for Disease Control and Prevention
Kathleen M. Rest, Ph.D., M.P.A. Acting Director	Rosemary Sokas, M.D., M.O.H. Associate Director for Science	National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention
Kenneth Olden, Ph.D. NTP Director	Christopher J. Portier, Ph.D. Director, Environmental Toxicology Program	National Institute of Environmental Health Sciences of the National Institutes of Health
Ruth Kirschstein, M.D. Acting Director (through April 2002) Elias A. Zerhouni, M.D., Director (beginning May 2002)	Vacant	National Institutes of Health
John L. Henshaw Assistant Secretary of Labor for Occupational Safety and Health	Steven F. Witt Director, Directorate of Health Standards Program	Occupational Health and Safety Administration of the U.S. Department of Labor
Thomas H. Moore Acting Chairman	Marilyn Wind, Ph.D. Deputy Associate Executive Director for Health Sciences	U.S. Consumer Product Safety Commission
Christine Todd Whitman Administrator	Vanessa Vu, Ph.D. Director, Science Advisory Board Staff Office	U.S. Environmental Protection Agency

*Membership as of May 15, 2002

ADDRESSING SCIENTIFIC AND REGULATORY OPPORTUNITIES

The NTP uses its goals to set priorities as it moves forward to improve the nation's ability to evaluate human health effects from exposures to chemical and physical agents of public health concern. Its vision, leadership, and commitment to the concept of good science for good decisions create an atmosphere that allows the program to be flexible and innovative in its approach toward addressing public health concerns related to chemical exposures at home and work and in our environment. The NTP has expanded its scope beyond cancer to include examining the impact of chemicals on non-cancer toxicities such as those affecting reproduction and development, and the immune, respiratory, and nervous systems. As part of this effort, the NTP Center for Evaluation of Risks to Human Reproduction was created.

The NTP recognizes that initiatives addressing critical knowledge gaps in toxicological evaluations offer the best opportunities for preventing environmentally mediated diseases. Therefore, the program's testing of chemicals is evolving to include more mechanism-based toxicology studies that focus on understanding the mode of actions of chemical agents. In recent years, the NTP has placed a greater emphasis on providing human context to the interpretation and understanding of toxicological information generated using animal or *in vitro* cell models. This is imperative in order to be at the forefront in research efforts to improve risk assessment methodologies for quantifying the sequence of events that starts with chemical exposure and ends with toxicity. Examples of activities it covers include:

- an increased effort to collect information on exposures, either environmental or occupational, and on substances or mixtures of concern;
- the increased application of mechanistic information and scientific judgment in the deliberations for listings in the Report on Carcinogens; and
- an enhanced effort to examine the merits of alternative testing methods that may give better information than current models using fewer animals, causing less pain or distress, and hopefully providing improved data to reduce uncertainties in risk assessments.

Nationally, the NTP rodent bioassay is recognized as the standard for identification of carcinogenic agents; however, the NTP continues to work to reduce the use of experimental animals and to develop and validate alternative testing methods. This effort has led to creation of the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM). The NTP will continue to work with the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM) under the NICEATM in promoting the development, validation and regulatory acceptance of new and revised alternative toxicological methods.

The strengthening of existing partnerships and the forging of new ones are important to achievement of the NTP's goals. Partnerships with sister Federal agencies are increasing and the NTP continues to collaborate with the private sector. Examples include co-sponsorship of numerous workshops, an interagency initiative in exposure assessment, establishment of the ICCVAM to oversee validation of alternative testing methods, and an interagency initiative to characterize occupational exposures. The NTP continues to support an effort to evaluate the phototoxicity of various compounds through the NTP Center for Phototoxicology at NCTR/FDA. In addition, the NTP is playing a role in providing toxicological assessments of water disinfection by-products and will provide this information to the U.S. Environmental Protection Agency (EPA) for its use in setting water standards.

Regulatory agencies make decisions for the protection of public health based on scientific information from multiple sources (*e.g.*, toxicology, human studies, and basic research). The NTP plays a critical role in providing needed scientific data, interpretations, and guidance concerning the appropriate uses of these data to regulatory agencies as well as other groups involved in health-related research. The program is committed to using the best science available in setting priorities for future studies and in designing, conducting, and interpreting the findings of those studies. The American people and government agencies at state and federal levels rely on the science base provided by the NTP in making credible decisions that will protect public health without unnecessarily increasing the regulatory burden on industry. Over the past two decades, the NTP has developed an increasingly interactive relationship with regulatory agencies and through this relationship has played an important, although indirect, role in shaping public health policy. The program maintains an objective, science-based approach in dealing with critical issues in toxicology and is recognized by many groups for its scientifically rigorous, objective, and open approach in the continuing dialogue concerning the appropriate application of scientific advances to applied toxicology research and testing.

COMMUNICATION AND PUBLIC OUTREACH

Maintaining open communications and ensuring dialogue with federal and state agencies, industry, stakeholders, academia, and the public are goals of the NTP. NTP advisory groups (see page 2) ensure regular scientific and public peer review and input. NTP conferences and workshops remain a priority and are designed to bring researchers, regulators, policy makers, and the public together to examine issues and achieve consensus on future directions in toxicology and risk assessment. Emphasis continues on ensuring broad dissemination of the results of NTP research and testing and communicating information about its evolving programs and priorities. The distribution of NTP testing and research results and its program plans, initiatives, announcements, press advisories, and publications occur through mailings, *Federal Register* announcements, and the world-wide-web that includes a subscription-based NTP listserve. The NTP homepage (http://ntp-server.niehs.nih.gov) offers access to information about the NTP, and links are available that detail and highlight ongoing and future initiatives, NTP centers, NTP documents, the Report on Carcinogens, and announcements.

On-line, searchable access and printed copies of NTP publications including the Report on Carcinogens, NTP Technical Reports and NTP Toxicity Reports are available through the *Environmental Health Perspectives* (EHP) at http://ehponline.org or 1-800-315-3010.

The NIEHS/NIH Central Data Management Office oversees distribution (upon request) of specific, chemical study information and printed NTP documents - the NTP Annual Plan, NTP Study Status Reports, pre-peer review copies of draft NTP Technical Reports, background documents for chemicals nominated to the NTP, and summaries of minutes from advisory committee meetings.

The NTP is interested in and welcomes stakeholder input into its programs and priorities. Nominations, inquiries, and comments from the public and other interested parties are welcome at any time. The NTP Liaison and Scientific Review Office at the NIEHS/NIH under the direction of Dr. Mary S. Wolfe serves as the focal point for receiving input to the program and for overseeing the distribution of information about programs, workshops, initiatives, etc.

NTP Liaison and Scientific Review Office
NIEHS/NIH
P.O. Box 12233, MD A3-01
111 T.W. Alexander Drive
Phone: (919) 541-0530
liaison@starbase.niehs.nih.gov

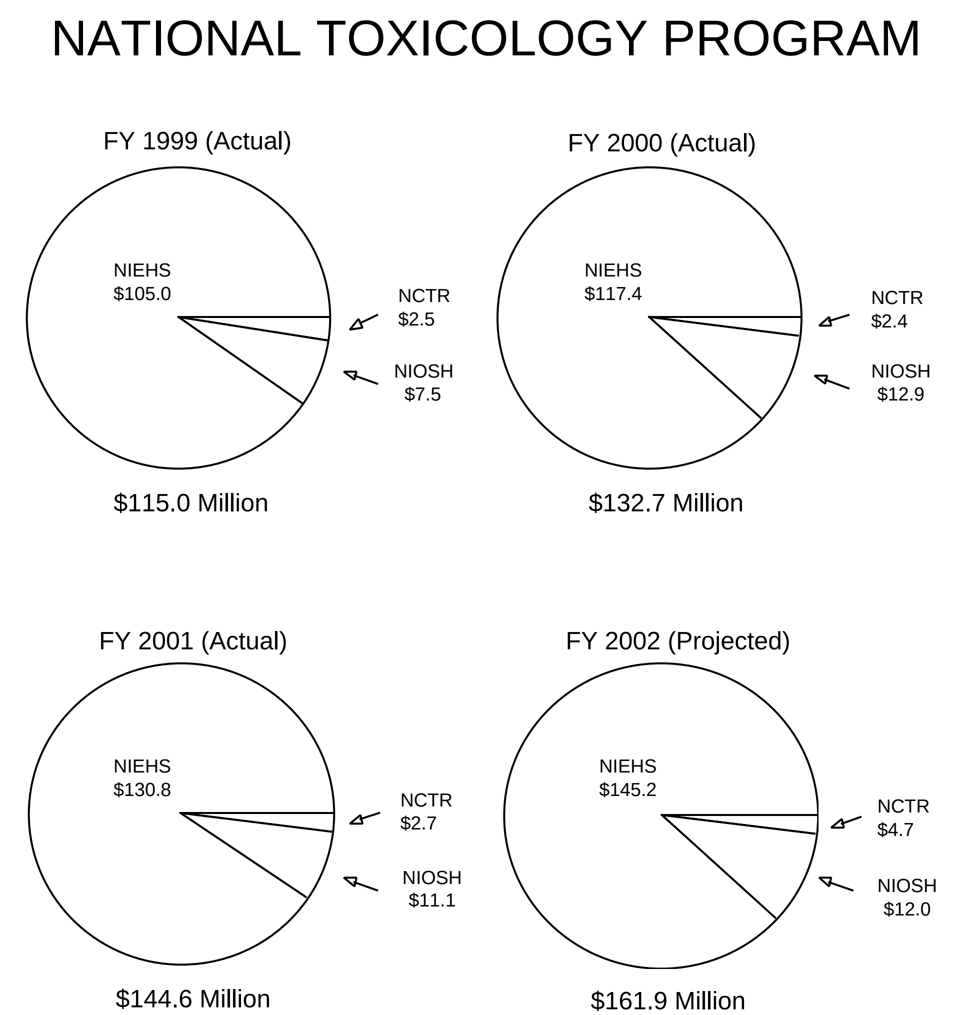
Central Data Management
NIEHS/NIH
P.O. Box 12233
79 T.W. Alexander Drive
Phone: (919) 541-3419
cdm@niehs.nih.gov

RESOURCES AND PLANNING

CURRENT AND PROJECTED RESEARCH CAPACITY

The NTP relies on voluntary allocations from the program's three core agencies (NIEHS/NIH, NCTR/FDA, and NIOSH/CDC) for supporting its various programs and initiatives. These allocations are specified following the determination of yearly appropriations. As shown in Figure 2, the actual allocations from the principals toward the NTP have steadily increased over the past three years (1999-2001) and are projected to provide a total funding level of $161.9M (direct plus indirect) in FY 2002. The NTP primarily conducts its research studies in-house at the core agencies or through contract laboratories, but also supports cooperative and/or collaborative agreements and small extramural grants (R03) with other federal agencies, academia and industry. Funds are also used to sponsor workshops and conferences and to produce and disseminate printed programmatic materials.

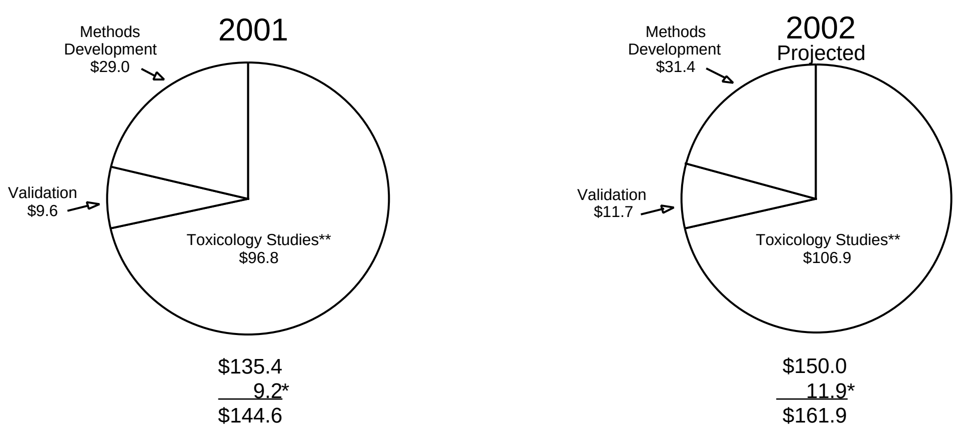
Figure 2. Agency Origin And Amounts of Funding (millions)



The NTP maintains an objective, science-based approach in dealing with critical issues in toxicology. The program continually sets its priorities to improve the nation's ability to evaluate human health effects from environmental exposures and focuses its resources on three major program activities: toxicology studies, methods development, and validation. As shown in Figure 3, approximately three fourths (71%) of the NTP's allocations (direct only) in FY 2001 were directed toward program activities in basic and applied research (toxicology studies) and a similar level of effort is projected for FY 2002 (71%). The NTP also has ongoing activities for the development and validation of improved research tools for carrying out its research studies. Approximately 29% of the NTP allocations are budgeted in FY 2002 for methods development and validation. These include NTP initiatives such as transgenic models, biomathematical modeling, and genomics. The directives in the 1993 NIH Revitalization Act regarding development of alternative methods that reduce, refine, or replace the use of animals in research provide legislative impetus for many of these efforts.

Figure 3. Funding Levels by Program Activities: FY 2001-2002

NATIONAL TOXICOLOGY PROGRAM (millions)



*Includes indirect operational support, etc.

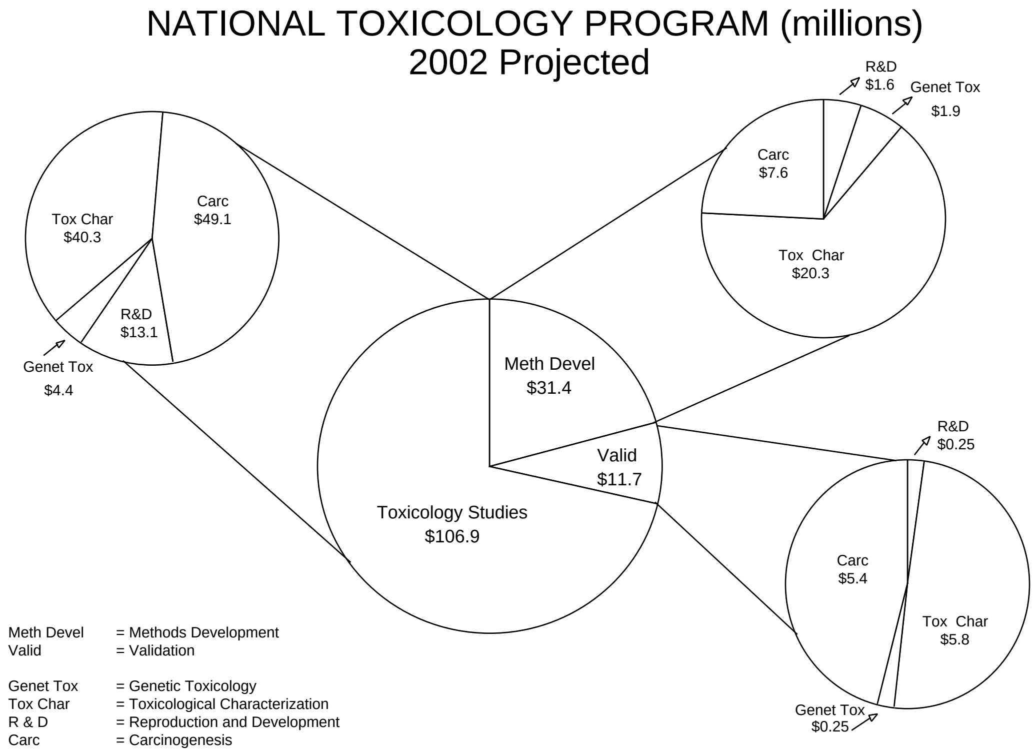
**Includes subchronic, chronic, immunotoxicology, chemical disposition, developmental, reproduction, and genetic toxicology studies.

Note: Excludes all Superfund and Acquired Immune Deficiency Syndrome activities.

The actual funding level (in million dollars) for the NTP in FY 2001 is provided for the three program areas: toxicology studies, methods development, and validation. This includes direct and indirect costs. The funding levels for FY2002 are projected. Funding represented combined allocations from the three core agencies.

Within each of the major program activities, the NTP targets multiple program areas broadly represented as genetic toxicology, toxicological characterizations (includes immunotoxicology, neurotoxicology, epidemiology, exposure assessment, and general toxicology), reproduction and development, and carcinogenesis. This involves conducting toxicological evaluations for cancer and non-cancer end points, generating data to strengthen the science base for risk assessment, developing and validating improved testing methods for targeted areas, and communicating with all stakeholders. Figure 4 shows the projected FY 2002 NTP allocations for each of these areas within individual program activities. For toxicology studies, the primary single focus remains on carcinogenesis although total projected funding for research on non-cancer end points (toxicological characterizations plus reproduction and development) studies is projected at 50%. Methods development and validation address strategies for both cancer and non-cancer end points. The majority of the funds for both methods development and validation ($20.3M or 65% and $5.8M or 50%, respectively) are allocated toward technologies that will improve the NTP's ability to characterize toxicities.

Figure 4. Funding Levels by Program Area



For each program activity (toxicology studies, methods development and validation), the projected allocated funds for FY 2002 are given for each major program area (genetic toxicology, toxicological characterizations, reproduction and development, and carcinogenesis.

In totality, the NTP is a comprehensive interagency research program whose core agencies are committed to providing resources for continuing the program's research efforts and for communicating the knowledge learned to all stakeholders, public and private. The program's efforts in toxicity testing and risk assessment are directed toward obtaining the best scientifically valid data that can be used by health, regulatory, and research agencies for making good decisions about potential human risk(s) from exposure to environmental toxicants. Toward that end, the NTP is continually evolving to remain at the cutting edge of scientific research and the development and application of technology.

EVOLVING PRIORITIES OF THE NATIONAL TOXICOLOGY PROGRAM

The NTP maintains a number of complex interrelated research and testing programs that provide unique data and knowledge used by health, regulatory, and research agencies to protect public health. These programs are well designed, are functioning successfully and exist to take advantage of opportunities afforded by advances in quantitative gene expression methods, transgenic and knockout models, exposure assessment, and other continuing and emerging scientific areas.

The NTP cancer bioassay program remains strong. In addition, the NTP has devoted programmatic and financial resources to two major efforts in the area of reproductive and developmental toxicology. One is the large study of endocrine disruptors underway at NCTR/FDA (see page 21), and the second is creation of the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR, see page 41). Children's health issues are of importance to the NTP and plans are underway to provide additional contract support to allow increased study of developmental immunotoxicology and developmental neurotoxicology. The NTP is continuing its support of activities associated with development, validation, and scientific review of new or revised alternative toxicological methods, especially those that will replace, refine, or reduce the use of animals in testing. The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NCEATM, see page 78) coordinates this effort and works closely with the NIEHS Interagency Coordinating Committee for the Validation of Alternative Methods to promote and communicate these activities. Efforts are of high priority that would strengthen intramural and extramural research activities in toxicogenomics, molecular biology, and molecular pharmacology. The NTP is a full partner in the newly established NIEHS National Center for Toxicogenomics (NCT, see page 30) and coordinates activities in this area with NTP member agencies.

Programmatic goals are constantly being updated and reevaluated. Ongoing efforts in FY 2002 will bring further clarity to the application of transgenic mouse models in the NTP research program. New transgenic model development and selection will continue with closer ties established to similar efforts at the National Cancer Institute/NIH. Efforts to evaluate transgenic animals for non-cancer toxicity end points have started. Toxicogenomics and proteomics will become better established as routine technologies in literally all disciplines within the NTP, and additional resources are being targeted to database development. These studies hold the promise of providing a true mechanistic basis for hazard identification through the use of short-term assays that can be practically applied over the broad range of agents to which humans are exposed. High priority research and testing programs, *i.e.*, herbal medicines, water disinfection by-products, radiofrequency radiation emissions from cellular phones, occupational exposures, will develop and expand.

Finally, the NTP will continue to expand a collection of activities designed to place research and testing results in better human health perspective. This encompasses such efforts as human exposure assessment, toxicokinetics and physiologically based pharmacokinetic modeling of bioassay findings, and interpretation of results in molecular epidemiology for use in human hazard identification (*e.g.*, Report on Carcinogens, CERHR). All of these initiatives need to be carried out in an arena of enhanced communication with all interested parties. The NTP has always drawn strength and credibility from its commitment to open information exchange and strict adherence to impartiality and rigorous scientific peer review. This will remain a central priority of the program in FY 2002 and in the years to come.

TOXICOLOGY AND CARCINOGENESIS EVALUATIONS

TOXICOLOGY AND CARCINOGENESIS EVALUATION PROCESS

Nomination

The NTP seeks to maintain a balanced research and testing program that provides data addressing a wide variety of issues important to public health. The NTP seeks the nomination of studies that permit the testing of hypotheses to enhance the predictive ability of future NTP studies, to address mechanisms of toxicity, or to fill significant gaps in the knowledge of the toxicity of chemicals or classes of chemicals. The NTP follows the principles for soliciting nominations listed in Table 2.

Table 2. Nomination Principles for NTP Studies

·	Chemicals found in the environment not closely associated with a single commercial organization
·	Biological or physical agents that may not be adequately evaluated without federal involvement
·	Commercial chemicals with significant exposure that were first marketed prior to current testing requirements or those that generate too little revenue to support further evaluations
·	Potential substitutes for existing chemicals or drugs that might not be developed without federal involvement
·	Substances that occur as mixtures for which evaluations cannot be required of industry
·	Chemicals that should be evaluated to improve the scientific understanding of structure-activity relationships and thereby help limit the number of chemicals requiring extensive evaluations
·	Emergencies or other events that warrant immediate government evaluation of a chemical or agent

The nomination process is open to the public. The NTP routinely solicits nominations at conferences and workshops; through the NTP newsletter, *Federal Register* notices, and NTP homepage (http://ntp-server.niehs.nih.gov); and from academia, federal and state regulatory and health agencies, industry, and labor unions, as well as from environmental groups and the general public. In addition, standing nomination committees within the NCI/NIH, FDA, NIOSH/CDC, and NIEHS/NIH routinely select and forward nominations to the NTP. The NTP also reviews toxic release inventories and exposure surveys [*e.g.*, National Health and Nutrition Examination Survey (NHANES) and National Human Exposure Assessment Survey (NHEXAS)] to identify chemicals of potential interest (Masten, Office of Chemical Nominations and Selection, NIEHS/NIH).

Selection

Nominations undergo a multi-step, formal process of review [Figure 5, Interagency Committee for Chemical Evaluation and Coordination (ICCEC), NTP Board of Scientific Counselors, and NTP Executive Committee]. During the entire process, the NTP works actively with regulatory agencies and interested parties to supplement information about nominated substances and to ensure that the nomination and selection process meets regulatory and public health needs. The ICCEC [1] plays a central role in recommending substances for NTP evaluation and coordinating NTP studies with other relevant agency activities. The ICCEC reviewed nominations to the NTP for toxicological testing at its May 2001 meeting and its recommendations are given in Table 3.

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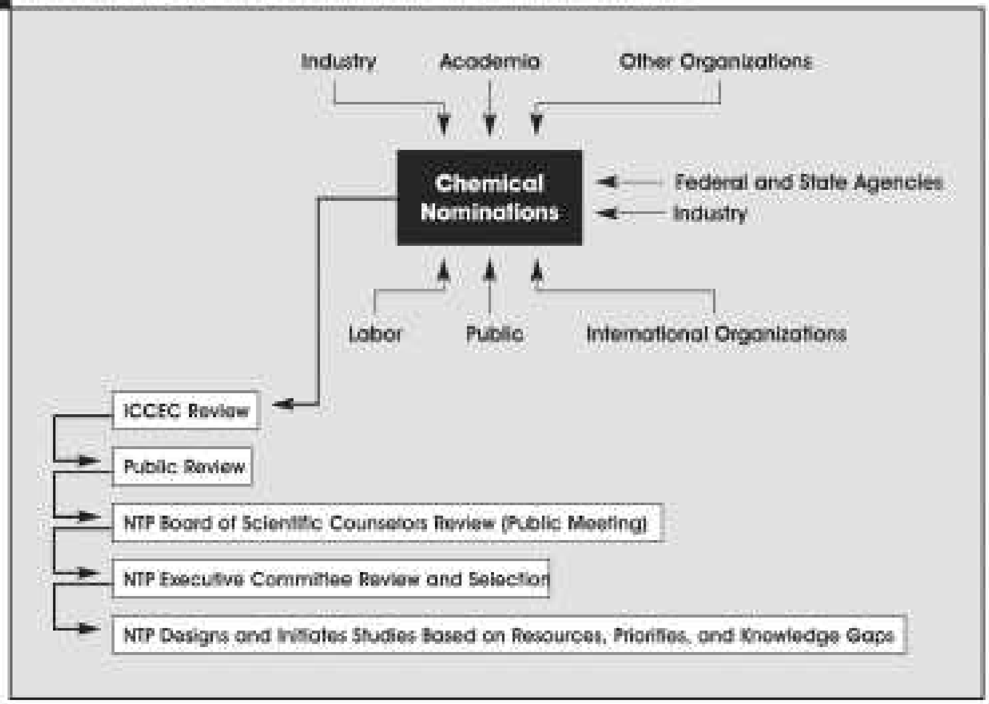
[1] Agencies represented on the ICCEC include: ATSDR, NCTR/FDA, NCI/NIH, NCEH/CDC, NIOSH/CDC, National Library of Medicine (NLM), Department of Defense (DOD), NIEHS/NIH, OSHA, EPA, and CPSC

Figure 5 **NTP Toxicology Study Nomination and Selection Process**



Public comments are solicited on nominated substances and those received are considered at all stages through study selection and design. At the final step of the formal process, the NTP Executive Committee reviews the substances and makes a final recommendation for acceptance of a nominated substance by the NTP. Table 4 lists substances approved by the Executive Committee at its June 2001 meetings.

The selection of a substance by the NTP Executive Committee does not automatically commit the NTP to its evaluation. The chemicals selected for study and the toxicology and cancer study designs are carefully considered to ensure that the dollars invested in NTP research are wisely spent. A chemical or study may be withdrawn if applicable research data or higher priority studies are identified or if a study proves impractical.

Table 3. Nominations for NTP Toxicological Studies Reviewed by the ICCEC[1] – May 2001

Substance (CAS No.)	Nominator	ICCEC Recommendation	Nomination Rationale; Other Information
Substances recommended by the ICCEC for testing			
Bladderwrack (68917-51-1) (84696-13-9)	NCI/NIH	Characterization of iodine content; subchronic toxicity testing with evaluation of reproductive parameters.	Significant human exposure through use as a dietary supplement; potential thyroid stimulation.
Cylindrospermopsin (14345-90-8)	NIEHS/NIH	Complete toxicological characterization including chronic toxicity and carcinogenicity testing.	Cyanobacterial toxin with potential for widespread human exposure through drinking water.
Epigallocatechin-3-gallate (989-51-5)	NCI/NIH	Genotoxicity testing; subchronic toxicity testing; consider testing green tea extract.	Major polyphenol in green tea and green tea extract dietary supplements.
2-Ethylhexyl-*p*-dimethylaminobenzoic acid (21245-02-3)	Private Individual	Subchronic toxicity and developmental and reproductive toxicity testing by the dermal route of exposure; phototoxicity and photocarcinogenicity testing.	High production volume chemical with industrial and consumer (sunscreen) uses.
Grape seed and pine bark extracts	NCI/NIH	Genotoxicity testing; subchronic toxicity testing; developmental and reproductive toxicity testing; select a standardized commercial pine bark extract for study.	Significant human exposure through use as a dietary supplement.
Metalworking fluids	NIOSH/CDC	*In vitro*, short-term *in vivo* and subchronic toxicity studies aimed at evaluating toxicity and carcinogenic potential of multiple commercial formulations; the ICCEC will make recommendations regarding further testing after reviewing the results of NTP preliminary studies.	High production volume; large number of occupationally-exposed workers; lack of carcinogenicity and chronic toxicity data.
Methyl tetrahydrofuran (96-47-9)	NCI/NIH	Genotoxicity testing; short-term toxicity testing; consider dermal and inhalation routes of exposure.	Increasing use in alternative fuels; suspicion of toxicity and carcinogenicity based on structure.
Polybrominated diphenyl ethers · Pentabromodiphenyl ether (technical) (32534-81-9) · Octabromodiphenyl ether (technical) (32536-52-0) · 2,2',4,4'-Tetrabromodiphenyl ether (5436-43-1) · 2,2',4,4',5-Pentabromodiphenyl ether (60348-60-9) · 2,2',4,4',5,5'-Hexabromodiphenyl ether (68631-49-2)	Private individuals and California Environmental Protection Agency	Subchronic toxicity, developmental neurotoxicity and chronic toxicity testing of selected individual congeners; no testing of technical mixtures	High production volume flame retardants; widespread human exposure occupationally and as environmental contaminants; persistent and bioaccumulative.
Substances for which no testing was recommended			
Apigenin (520-36-5)	NCI/NIH	No studies due to insufficient toxicity and exposure potential.	Naturally occurring flavonoid with potential oxidant and estrogenic activity.
Dibenzofuran (132-64-9)	NCI/NIH	No studies due to low commercial production volume and low potential for human exposure.	Potential widespread human exposure as an environmental contaminant.

Substance (CAS No.)	Nominator	ICCEC Recommendation	Nomination Rationale; Other Information
Diphenolic acid (126-00-1)	NCI/NIH	No studies due to low commercial production volume and low potential for human exposure.	Industrial chemical with potential for increasing use; suspicion of toxicity.
Testing recommendation deferred pending receipt and consideration of additional information			
n-Butyl bromide (109-65-9)	NCI/NIH	Defer pending information regarding manufacturers' voluntary testing plans.	Industrial chemical with significant production volume and human exposure potential; suspicion of carcinogenicity.
Methyl soyate (67784-80-9)	NCI/NIH	Defer pending information about toxicity data development plans through existing or future regulatory programs.	Increasing production volume as an alternative fuel (biodiesel).

[1] ICCEC – Interagency Committee for Chemical Evaluation and Coordination

Table 4. Toxicological Studies Recommended by the NTP Executive Committee – June 2001

Substance (CAS No.)	Nominator	Study Recommendations	Nomination Rationale; Other information
Substances Recommended for Testing			
Aluminum complexes found in drinking water · Aluminum fluoride (7784-18-1) • Aluminum citrate (31142-56-0)	EPA NIEHS/NIH	Long-term drinking water studies to address pharmacokinetics, neurotoxicity, bone development, and reproductive and developmental toxicity. Consideration for testing in transgenic models of neurodegenerative disease.	A better understanding of pharmacokinetics and toxicity of aluminum species occurring in drinking water is needed.
Bilberry fruit extract (84082-34-8)	NCI/NIH	*In vitro* and *in vivo* genotoxicity testing.	Widespread human exposure through use as a dietary supplement.
Black cohosh (84776-26-1)	NCI/NIH NIEHS/NIH	Subchronic toxicity testing in young and female animals; two-generation reproductive and developmental toxicity testing.	Widespread human exposure as a dietary supplement; reported estrogenic activity.
Blue-green algae (dietary supplement and selected toxins)	NCI/NIH	Subchronic toxicity testing and neurotoxicity studies of commercial substances; consider follow-up studies of cyanobacterial toxins pending blue-green algae and microcystin-LR studies.	Widespread human exposure through drinking water and via contamination of algal dietary supplements.
Cefuroxime (55268-75-2)	FDA	Genotoxicity testing	Prescription drug with widespread and potentially long-term use.
Clarithromycin (81103-11-9)	FDA	Genotoxicity testing	Prescription drug with widespread and potentially long-term use.
D&C Red No. 27 (13473-26-2) and D&C Red No. 28 (18472-87-2)	FDA	*In vitro* percutaneous absorption testing; photocarcinogenicity testing pending absorption study results.	Approved colorings for drugs and cosmetics that can lead to DNA damage.
N,N-Dimethyl-*p*-toluidine (99-97-8)	NCI/NIH	Subchronic toxicity testing pending review of industry test plans and/or data developed under EPA's HPVC Challenge Program.	High production volume chemical with potential for widespread human exposure.
Lemon oil (8008-56-8)and Lime oil (8008-26-2)	FDA	Phototoxicity testing; Photocarcinogenicity testing pending phototoxicity testing study results.	Widespread consumer exposure as a fragrance component.
Local anesthetics that metabolize to 2,6-xylidine or *o*-toluidine · Bupivacaine (38396-39-3)	Private individual NIEHS/NIH	Short-term *in vitro/in vivo* mechanistic studies for carcinogenic metabolite formation and genotoxicity of representative anesthetic compounds.	Widespread clinical use and human exposure; potentially metabolized to carcinogenic and neurotoxic intermediates.

Substance (CAS No.)	Nominator	Study Recommendations	Nomination Rationale; Other information
· Prilocaine (721-50-6)			
Microcystin-LR (101043-37-2)	NIEHS/NIH	Toxicokinetic, subchronic, reproductive toxicity, chronic, toxicity and carcinogenicity studies; consider Medaka fish model studies.	Cyanobacteria and their toxins are drinking water contaminants with potentially widespread human exposure.
Organotins occurring in drinking water • Monomethyltin trichloride (993-16-8) • Dimethyltin dichloride (753-73-1) • Monobutyltin trichloride (1118-46-3) • Dibutyltin dichloride (683-18-1)	EPA NIEHS/NIH	Long-term single chemical and binary mixture drinking water studies that address pharmacokinetics, neurotoxicity, immunotoxicity, and reproductive and developmental toxicity.	Drinking water contaminants with potentially widespread human exposure.
All-*trans*-retinyl palmitate (79-81-2)	FDA	Phototoxicity and photocarcinogenicity testing.	Widespread use in cosmetic products.
S-Adenosylmethionine (29908-03-0)	NCI/NIH	*In vitro* genotoxicity testing; sub-chronic toxicity pending genotoxicity study results.	Widespread exogenous human exposure through use as a dietary supplement.
Senna (8013-11-4)	FDA	Carcinogenicity testing in p53 transgenic model.	Data are needed to complete safety evaluation of stimulant laxatives.

Evaluation

In carrying out its mission, the NTP provides toxicological evaluations on substances of public health concern. Unfortunately the NTP can initiate bioassays to characterize potential carcinogenicity of substances on only a small fraction of the thousands of chemicals for which there is little or no information. Many more chemicals are also studied to assess a variety of non-cancer health-related effects including, but not limited to, reproductive and developmental toxicities, immunotoxicity, neurotoxicity, and genotoxicity. Other biologic end points are often evaluated, such as quantifying the disposition and excretion of substances, identifying and correlating biochemical markers with exposure and metabolism, and examining genetic polymorphisms in human drug metabolizing enzymes to understand the susceptibility of individuals and populations to xenobiotic-induced toxicity.

An NIEHS/NTP project review committee reviews and evaluates a study's project plan (*e.g.*, design, methods, hypothesis, etc.) and proposes the vehicle for execution (*e.g.*, grant, contract, etc). The toxicological evaluation is generally conducted through repeated administration of a substance to groups of laboratory animals for variable periods of time up to two years. Many of the short-term studies are designed to provide dose-setting information for instigating chronic evaluations and to address specific deficiencies in the toxicology database. The adverse health effects from short- or long-term exposures of different dose levels of the substance are evaluated clinically, by histopathology, and by a variety of toxicology end points through comparison with groups of animals not administered the substance. Many substances are also studied using protocols specifically designed to address issues pertaining to the mechanism by which a substance causes a particular toxic outcome(s). General information about the objectives and procedures of NTP study protocols is available on the

NTP web site (http://ntp-server.niehs.nih.gov, see NTP Study Information). Support activities at the core agencies facilitate conduct of these evaluations and include:

- animal production and care (Witt, NCTR/FDA; Rao, NIEHS/NIH)
- archives (Maronpot, NIEHS/NIH)
- biological monitoring and health assessment (DeBord, NIOSH/CDC)
- chemistry/biochemistry (Turesky, NCTR/FDA; Smith/Collins/Overstreet, NIEHS/NIH; Snawder, NIOSH/CDC)
- chemical disposition contracts (Cunningham, NIEHS/NIH); chemical metabolism (Burka, NIEHS/NIH)
- clinical pathology (Travlos, NIEHS/NIH)
- genetic toxicity testing (Caspary, NIEHS/NIH)
- information retrieval and analysis (Tatken, NIOSH/CDC; Wright, NIEHS/NIH)
- information systems and central files (Eastin, NIEHS/NIH), database management – CHEMTRACK, TDMS and LDAS (Rowley, NIEHS/NIH), NTP web page (Soward, NIEHS/NIH)
- mass spectrometry (Tomer, NIEHS/NIH)
- microbiology (Cerniglia, NCTR/FDA; Rao, NIEHS/NIH)
- pathology (Hailey/Herbert, NIEHS/NIH; Salomon, NIOSH/CDC)
- quality assurance (Reed, NCTR/FDA; Bristol, NIEHS/NIH; Pringle, NIOSH/CDC)
- statistical services (Kodell, NCTR/FDA; Haseman/Dunson, NIEHS/NIH; Krieg, NIOSH/CDC)
- study coordination/oversight (Jackson, NCTR/FDA; Bucher, NIEHS/NIH; Toraason, NIOSH/CDC)
- toxicogenomics: microarray (Afshari, NIEHS/NIH); proteomics (Merrick, NIEHS/NIH)
- technical report preparation (Alden, NIEHS/NIH; Schulte, NIOSH/CDC)
- toxicology and carcinogenicity testing (Orzech/Roycroft, NIEHS/NIH)
- toxicity testing (Chhabra/Vallant, NIEHS/NIH)
- transgenic mouse colonies (Stasiewicz, NIEHS/NIH)

The NTP carries out toxicology and carcinogenesis research through two primary mechanisms: laboratory studies conducted in contract laboratories and in-house studies conducted at its core agencies: NIEHS/NIH, NCTR/FDA, and NIOSH/CDC. In addition, the NTP leverages resources through cooperative and/or collaborative agreements with other Federal agencies, academia, and industry.

The NIEHS/NIH Division of Extramural Research and Training supports research on methods development and in collaboration with the NTP supports investigator-initiated research to provide data to aid in defining the mechanisms of action of agents under study by the NTP. Currently research under the NIH R03 Small Grant mechanism is supporting investigator-initiated research on the mechanism of toxicity/carcinogenicity of water disinfection by-products (see page 18). A new initiative is being developed to encourage extramural investigator involvement in NTP studies employing specific genetically altered or transgenic mice, as well as studies examining gene expression and protein changes (genomics and proteomics).

The NIEHS/NIH is supporting an interagency agreement with the Lawrence Livermore National Laboratory in collaboration with the University of California at Berkeley to develop, maintain, update, and upgrade a comprehensive database of laboratory animal carcinogenicity study results taken from the literature, including carcinogenesis studies conducted by the

NTP. The Carcinogenic Potency Database contains the results of chronic, long-term animal cancer tests, positive and negative.

In addition to toxicology research of compounds and exposures, the NTP supports the development of new techniques and methods for improving the ability to identify and assess potential environmental toxicants and the development and validation of novel and alternative testing methods that will reduce, replace, or refine animal use. The NTP also supports development of improved statistical methods for toxicology studies. Improvements are being made in the analysis of tumor multiplicity data and in risk assessment and testing in toxicology studies that measure multiple end points (Dunson, NIEHS/NIH). One project focuses on development of new statistical methods for dealing with tumor incidences and dose-related trends in tumor rates (Dinse, NIEHS/NIH) and another is identifying and evaluating sources of variability in response in rodent studies. Such information may permit modifications in study protocols to lessen the impact of these potentially confounding factors on the interpretation of experimental findings (Haseman, NIEHS/NIH).

Review

The results of toxicology and carcinogenesis studies undergo rigorous peer review. These findings are published as NTP Technical Reports and may also be published in peer-reviewed scientific journals. The Technical Reports Review Subcommittee of the Board (see Advisory Committees, page 2) evaluates the technical reports in an open, public meeting. Candidates for peer review in 2002 and 2003 are listed in Table 5. Abstracts of the NTP Technical Reports series and the NTP Toxicity Report series are posted on the NTP web site and hardcopies and PDFs are available from *Environmental Health Perspectives* (see page 5). Both report series are also catalogued in MedLine.

Table 5. Candidate Chemicals for Peer Review

Chemical	Technical Report No.	Information
September 2002		
trans-Cinnamaldehyde	TR 514	Used as a flavoring for foods and beverages; the principal ingredient of cinnamon oil.
Decalin	TR 513	Used as an industrial solvent for naphthalene, fats, resins, oils, and waxes, as a substitute for turpentine, and as a constituent of motor fuels and lubricants.
Dipropylene glycol	TR 511	Used in air and room fresheners, household cleansers, cosmetic formulations, auto paints and antifreeze.
Elmiron	TR 512	Used in treatment of thrombosis and hyperlipidemia and for relief of urinary bladder pain associated with interstitial cystitis.
Pentaerythritol triacrylate	TR 517	Representative multifunctional acrylate assessed in a transgenic mouse model.
Trimethylolpropane triacrylate	TR 516	Representative multifunctional acrylate assessed in a transgenic mouse model.
Urethane + Ethanol	TR 510	The major human exposure to urethane is in fermented foods and beverages. Urethane was studied in combination with ethanol because of widespread exposure through alcoholic beverages.
2003		
2-Methylimidazole		
3,3',4,4',5-Pentachlorobiphenyl		
Propylene glycol mono-*t*-butyl ether		
Stoddard solvent (Type IIc)		
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)		
Triethanolamine		

HIGHLIGHTED CURRENT NTP INITIATIVES

The NTP has a broad mandate to provide toxicological characterizations for chemicals and agents of public health concern and strives to balance the selection of chemicals for study. This has resulted in a diverse research program, but with emphasis on synthetic industrial chemicals, pesticides, various pharmaceuticals, metals, and food additives. The following section highlights some current NTP initiatives: several areas that have received inadequate attention in the past, *i.e.*, photoactive chemicals, contaminants of finished drinking water, endocrine disrupting agents, and certain occupational exposures; and research addressing potential safety issues associated with herbal medicines, radiofrequency radiation emissions from cellular telephones, hexavalent chromium and DNA-based therapies. In general, these initiatives are broad-based and include various health-related end points.

Radiofrequency Radiation Emissions from Cellular Phones

Over 100 million Americans currently use wireless communication devices with thousands of new users added daily. Personal (cellular) telecommunications is a rapidly evolving technology that uses microwave radiation to communicate between a fixed base station and mobile user. Most systems employ a hand-held cellular telephone where the radiation antenna is close to the user's head. Cellular phones and other wireless communication devices are required to meet the radiofrequency radiation exposure guidelines of the Federal Communication Commission. These guidelines are based on protecting the user from acute injury from thermal effects produced by radiofrequency radiation. Current data are insufficient to draw definitive conclusions concerning the adequacy of these guidelines for protecting against potential adverse effects of chronic exposure.

Studies in laboratory animals are considered crucial for understanding whether exposure to radiofrequency radiation may pose a danger to human health. Other research groups are performing several long-term animal studies addressing this issue. In addition, the NTP plans to conduct additional laboratory research to help clarify any potential health hazard for the U.S. population. The NTP is working with technical experts from the National Institute of Standards and Technology (NIST) to test the suitability of various radiofequency radiation exposure systems for use in these studies (Melnick, NIEHS/NIH).

NTP staff are working with radiofrequency experts at the NIST to evaluate studies being planned or underway by a consortium of European investigators under the auspices of the European Union and by investigators at the Cancer Research Center of the European Ramazzini Foundation of Oncology and Environmental Sciences Commission (Melnick/Roycroft, NIEHS/NIH).

Safe Drinking Water Program

It is estimated that more than 200 million Americans use municipally treated drinking water, so the availability of safe drinking water is of enormous public health significance. Although chlorination is considered one of the major public health advances of the twentieth century, chemical disinfection by-products (DBPs) of chlorination or other disinfection processes may cause health problems such as cancer. In addition, there are agents found naturally in water or are present by contamination of public water systems that may pose a threat to public health.

The U.S. Environmental Protection Agency (EPA) has responsibility to set water standards for DBPs. To provide scientific data for setting sound water quality standards, the NTP is collaborating with the EPA on a research program to assess potential risks from human exposure to DBPs. This program includes a systematic, mechanism-based, toxicological evaluation of DBPs focusing on reproductive toxicity, immunotoxicity, neurotoxicity, and carcinogenicity. Selection of DBPs for study is based on their presence in drinking water, occurrence with different disinfection processes, chemical structures, and representation of several DPB classes: trihalomethanes, haloacetic acids, and haloacetonitriles. DBPs currently under study by the NTP are listed in Table 6.

Table 6. Water Disinfection By-Products under Study

Chemical	NTP Studies (ongoing)
Bromochloroacetic acid	Chronic testing
Bromodichloromethane	Subchronic testing, chronic testing, transgenic models, toxicokinetics
Chloramine	Immunotoxicity
Chloroform	Immunotoxicity
Dibromoacetic acid	Subchronic testing, chronic testing, immunotoxicity, neurotoxicity
Dibromoacetonitrile	Chronic testing, neurotoxicity, chemical disposition
Dichloroacetic acid	Immunotoxicity
Sodium bromate	Reproduction and development
Sodium chlorate	Chronic testing
Sodium chlorite	Immunotoxicity

NTP research under this program is broad in scope with some studies being conducted at NIEHS/NIH and others being done through agreements with the U.S. Army for fish studies and EPA for immunotoxicity and neurotoxicity investigations. A collaborative government/industry partnership is in place where the NIEHS/NIH supplies transgenic animals and pathology expertise for industry inhalation studies on bromodichloromethane. The NTP is also involving the extramural research community through grant (R03) support of hypothesis-based mechanistic studies on DBPs and is working closely with the American Water Works Association Research Foundation (AWWARF) sharing protocols and research plans and making them aware of ongoing research activities. Some of the AWWARF's own research awards are being designed to complement activities of the NTP and EPA.

Besides DBPs, a complex array of agents may occur naturally (*e.g.*, arsenic, aluminum), as a result of contamination (*e.g.*, methyl tertiary butyl ether, pesticides, organotins), or with environmental changes (*e.g.*, algal blooms resulting in cyanobacterial toxins in surface waters). The NIEHS/NIH and EPA are prioritizing toxicology studies on several of these agents including aluminum complexes, organotins, and the two most common cyanobacterial toxins: microcystin-LR and cylindrospermopsin, for further evaluation by the NTP (Melnick, NIEHS/NIH).

Phototoxicology

As a result of the public's increasing exposure to ultraviolet (UV) radiation from sunlight and other sources (*e.g.*, tanning booths), the NTP has initiated research to learn what toxic effects, if any, might occur from such exposures. The NTP is coordinating an effort between the NIEHS/NIH and NCTR/FDA to study the phototoxicology and photocarcinogenicity of substances nominated to the NTP including those of high priority to the FDA. In general, these studies investigate the effects on gene expression, toxicity, and carcinogenicity of sunlight combined with either topically or systemically applied substances in the SKH-1 hairless mouse. Much of this research is being carried out at the NTP Center for Phototoxicology (NCP, see page 39).

Phototoxicology studies are in progress at the NCP for topically applied chemoexfoliating acids (α- and β-hydroxy acid) and aloe vera. The α- and β-hydroxy acids are included in many cosmetics. In some cases, they are added to correct or improve the appearance of "sun-aged" skin so that it appears smoother and less wrinkled. The impact on skin cancer from their continuous use in combination with exposure to sunlight is not known. Studies underway at the NCP are using glycolic acid and salicylic acid as representative α- and β-hydroxy acids, respectively (Howard, NCTR/FDA). The NCP is also investigating the effect on acute toxicity and photocarcinogenesis of topically applied plant fractions of the Aloe vera plant in combination with simulated sunlight. Numerous products including cosmetics and dietary supplements include portions of the Aloe vera plant (Boudreau, NCTR/FDA). Additional chemicals in the phototoxicity program include retinyl palmitate (a Vitamin A derivative in cosmetics), Padamate O (lemon and lime oils), D&C 27 and 28 (cosmetics), tattoo ink chemicals, and fluorescein-based dyes (Howard, NCTR/FDA).

Animal models that suffice as surrogates to test the role of specific UV wavelengths and chemicals in the development of human malignant skin melanoma do not exist at this time. As a result, the effects on melanoma development of chemicals combined with exposure to sunlight are not understood. The NCP is investigating the suitability of a transgenic mouse [TP-*ras* (+) p16/INK4a (+/-)] as a surrogate animal for studying melanoma formation (Howard, NCTR/FDA).

Ultraviolet (UV) radiation from the sun is the major environmental factor responsible for a high incidence of non-melanoma skin cancer. Occupational exposures in predominately outdoor professions may place those workers at increased risk of skin damage and cancer. The molecular mechanisms involved in UV-induced toxicity and carcinogenesis are not fully understood. In response to a request from the U.S. Congress, NIOSH/CDC is addressing potential health risks associated with occupational exposures to UV radiation. Specific aims include examining alterations in gene expression induced by UV radiation, understanding the involvement of signal transduction pathways in this gene alteration, studying the role of this gene alteration in cancer development, elucidating the mechanisms for UV-induced skin cancer, and identifying natural antioxidant compounds that may prevent UV-induced diseases. Transgenic mouse epidermal cell lines as well as transgenic mouse models are being used. Preliminary results provide molecular evidence that fresh apple extract contains inhibitory compound(s) for UVB-induced signal transduction and tumor promotor-induced cell transformation. Further studies will focus on identification of the active compounds in the extract and the mechanism(s) of action (Ding, NIOSH/CDC).

Herbal Medicines

Medicinal herbs are among our oldest medicines, and their increasing use in recent years is evidence of a public interest in alternatives to conventional medicine. Approximately one-third of the U.S. population is believed to use some form of alternative medicine, including herbal remedies. The use of herbal medicines and other dietary supplements has increased substantially since passage of the 1994 Dietary Supplement Health and Education Act. Although approximately 1,500 botanicals are sold as dietary supplements or ethnic traditional medicines, herbal formulations are not subjected to FDA pre-market approval to ensure their safety or efficacy.

The NTP is planning or conducting research (Table 7) on several medicinal herbs and compounds found in herbs that focus on carcinogenicity, reproductive toxicity, neurotoxicity, immunotoxicity, and toxic effects associated with acute exposures to high doses and chronic exposures to low doses (Burka, NIEHS/NIH; Boudreau, NCTR/FDA).

Table 7. Herbs and Herbal Ingredients under Study by the NTP

Herb or Ingredient	NTP Studies (on-going or planned)	Information
Aloe vera gel	Skin painting/UV studies underway. Protocol for drinking water study are complete.	Used as both a dietary supplement and component of cosmetics. Used as a treatment for minor burns. Increasingly consumed orally in “health drinks.”
Black cohosh	Study being planned.	Used to treat symptoms of pre-menstrual syndrome (PMS), dysmenhorrea, and memopause.
Black walnut extract	Metabolism and disposition studies on juglone; design stages for pre-chronic and subchronic studies. Immunotoxicology studies to determine if black walnut extract is has the potential to induce contact hypersensitivity.	Found in hair dye formulations and walnut oil stain. Juglone is a major constituent.
Echinacea purpurea extract	Immune function studies in B6C3F1 mice. Modulation of autoimmune responses in NZB/W mice, a model for human Systemic Lupus Erythmatosis.	A commonly used medicinal herb in the United States. Used to treat colds, sore throat, and flu.
Ginkgo biloba extract	Subchronic testing underway.	Among the five or six most frequently used medicinal herbs. Ginkgo fruits and seeds have been used medicinally for thousands for years. The extract of green-picked leaves has increasing popularity in the United States. Ginkgo biloba extract promotes vasodilatation and improved blood flow and appears beneficial, particularly for short-term memory loss, headache, and depression.
Ginseng and Ginsenosides	Pre-chronic studies will start in FY 2002.	Fourth most widely used medicinal herb used in this country. Ginsenosides are thought to be the active ingredients in ginseng. Used as a laxative, tonic, and diuretic. Ginseng has been associated with adverse health effects.
Goldenseal	Subchronic study will start in FY 2002.	Second or third most popular medicinal herb used in this country; traditionally used to treat wounds, digestive problems, and infections. Current uses include as a laxative, tonic, and diuretic.
Grape seed extract and pine bark extract	Study design underway; metabolism and distribution studies of kawain, a kava component, underway.	Widely used herbals. The extracts are used to promote health of the cardiovascular system.
Kava kava	Design of pre-chronic, subchronic, and chronic studies is complete.	Reported to be the fifth most widely used medicinal herb. Has psychoactive properties. Sold as a calmative and antidepressant.
Milk thistle extract	Genetic toxicity testing of milk thistle extract, milk thistle tea, and individual components: silybin and silymarin. Subchronic studies will be conducted this year.	Used to treat depression and several liver conditions, including cirrhosis and hepatitis, and to increase breast milk production.
Pulegone	Subchronic studies will be completed in FY 2002; metabolism and disposition studies are ongoing.	A major terpenoid constituent of the herb, Pennyroyal, is found in lesser concentrations in other mints. Pennyroyal has been used as a carminative insect repellent, emmenagogue, and abortifacient. Pulegone has well-recognized toxicity to the liver, kidney, and central nervous system.
Thujone	Subchronic studies will be completed in FY2002.	Terpenoid found in a variety of herbs, including sage and tansy, and in high concentrations in wormwood. Suspected as the causative toxic agent associated with drinking absinthe, a liqueur flavored with wormwood extract.

Hexavalent Chromium

Chromium is a naturally occurring element present in various valence states. Trivalent chromium is an essential nutrient and chromium occurs most commonly in nature in this state. Hexavalent chromium compounds are the next most stable form; however, they rarely occur naturally and are typically associated with industrial sources.

Based upon concern by a number of California legislators, the California Environmental Protection Agency, and the California Department of Health Services, the NTP is studying the carcinogenic potential of hexavalent chromium administered in drinking water. Hexavalent chromium is an established human carcinogen in certain occupational settings, presumably as a result of inhalation exposure. There is uncertainty, however, regarding the long-term consequences of exposure to hexavalent chromium compounds in the water supply. The currently available data on the chronic toxicity and carcinogenicity of hexavalent chromium after oral exposure are largely inadequate to establish or characterize any hazard. The NTP studies will include both short- and long-term administration of hexavalent chromium as sodium dichromate dihydrate in drinking water to laboratory animals, as well as studies to characterize hexavalent chromium's tissue absorption. Data from the absorption study and outlines of the designs of all studies on hexavalent chromium are accessible on the NTP web site (http://ntp-server.niehs.nih.gov). The NTP is planning a public meeting in July 2002 to discuss the data obtained from the absorption and short-term studies and the proposed study designs for 2-year rodent cancer studies (Abdo, NIEHS/NIH).

Endocrine Disrupting Agents

Endocrine disruptors are naturally occurring or man-made substances that may mimic or interfere with natural hormones in the body. Endocrine disruptors may turn on, shut off, or modify signals that hormones carry and thus affect the normal functions of tissues and organs. The NTP is involved in several efforts to strengthen the science base within this field.

The NIEHS/NIH and the NCEH/CDC are collaborating to quantify approximately 70 chemicals found in human blood or urine that are considered endocrine-disrupting agents, including phthalates and phytoestrogens. The biological samples are collected as part of the National Health and Nutrition Examination Survey (NHANES), which includes men and women from a range of age, socioeconomic, and ethnic groups. The first edition of CDC's *National Report on Human Exposure to Environmental Chemicals*, released in 2001 presented levels of 27 environmental chemicals measured in NHANES samples including phthalate metabolites. Continued development of this interagency exposure initiative will focus on other NTP priority exposures, such as herbal medicines and drinking water disinfection by-products, to facilitate sound scientific evaluations of agents of priority for public health (Masten, NIEHS/NIH). This study complements the external scientific peer review conducted by the NTP Center for the Evaluation of Risks to Human Reproduction on the potential reproductive and developmental toxicity of phthalate esters (CERHR, see page 41).

Endocrine-disrupting chemicals are of interest to the FDA, and through an interagency agreement the NIEHS/NIH supports toxicology studies being conducted at the NCTR/FDA (Table 8). Chemicals under study include the phytoestrogen genistein, the pesticides vinclozolin and methoxychlor, the drug ethinyl estradiol, and the industrial chemical nonylphenol. These studies assess effects on reproduction, development of hormone-sensitive organs, and cancer in rodents over multiple generations (Delclos, NCTR/FDA). Behavioral and immunological end points are also being evaluated (behavioral – Ferguson, NCTR/FDA; neurotoxicity – Scallet, NCTR/FDA). The NCTR/FDA scientific staff is also interested in evaluating neuroanatomical and neurobehavioral end points associated with exposure to endocrine disrupting chemicals. Studies are underway to determine the neurohistological structure of sexually dimorphic regions of the hypothalamus with companion studies designed to assess male and female reproductive behaviors known to reflect neurotoxicological alterations, as related to endocrine active chemical insult. These are critical parallel studies to the multigeneration toxicology studies (Slikker, NCTR/FDA). Additional studies are also investigating the role of the p53 tumor suppressor gene in evaluating genistein (Morris, NCTR/FDA, see page 54).

Table 8. Endocrine Disrupting Agents under Study

Chemical	NTP Studies (ongoing)
Ethinyl estradiol	Reproduction/development, immunotoxicity, transgenic models, neurotoxicity
Genistein	Reproduction/development; cancer, immunotoxicity, neurobehavior
Methoxychlor	Reproduction/development, neurotoxicity, cancer
Nonylphenol	Reproduction/development, neurobehavior, immunotoxicity
Vinclozolin	Reproduction/development, immunotoxicity, neurotoxicity

As required by the 1996 Food Quality Protection Act, the EPA is in the process of choosing appropriate assays to screen endocrine-active agents and develop standardized, validated protocols for those assays. The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM, see page 78) is involved in preparing background documents for the EPA on current *in vitro* methods for assessing androgenic and estrogenic activities of chemicals. A peer review of these methods is planned for May 21-22, 2002.

The NIEHS/NIH and the NCTR/FDA are examining the cellular and molecular mechanisms through which natural and synthetic estrogenic compounds might interact with target tissues (*e.g.*, developing genital tract, gonads, liver, and mammary gland) and cause permanent alterations that potentially could affect sexual differentiation or induction of hormone-sensitive cancers (*e.g.*, cervix, uterus, vagina, breast, testis, and prostate). Diethylstilbestrol is being used as the model compound for comparison of effects with naturally occurring substances (genistein found in soy), drugs (Tamoxifen), and pesticides (methoxychlor) (Newbold, NIEHS/NIH; Delclos, NCTR/FDA). Other studies include identifying stem cells in target tissues, investigating the role of the estrogen receptor in induction and/or progression of neoplasms, identifying potentially altered growth factor pathways, and defining markers of pre-neoplasia. This group is also interested in developing assays to determine estrogenicity of environmental chemical sensitive markers of exposure (Newbold, NIEHS/NIH).

Studies at NIOSH/CDC are exploring *in vitro* how exposure to occupational chemicals impacts normal testicular function at different stages of maturation/development (fetal/neonatal, prepubertal, and adult). Primary cultures of rat Leydig (synthesize testosterone) and Sertolli (role in regulating spermatogenesis) cells from animals at different ages following exposure are being used. These studies target the testing of endocrine – disrupting chemicals (industrial surfactant additive – octylphenol and pesticides – methoxychlor and vinclozolin), which are reported to alter normal functioning of endogenous steroids (estrogens and androgens). Results from initial studies demonstrate age-dependent responses to chemical exposures. Future studies will focus on establishing possible mechanism(s) of action and comparing *in vitro* and *in vivo* responses (Murono, NIOSH/CDC).

Two NIEHS/NIH epidemiology studies are examining the potential effects of endocrine disrupting agents in human populations. One project is investigating the impact of exposure to hormonally active compounds on sexual differentiation in offspring (Rogan, NIEHS/NIH, see page 68). Another project is focusing on the effects of early-life organochlorine exposure and includes examining the relationship between *in utero* exposure to polychlorinated biphenyls (PCBs) and neonatal thyroid function (Longnecker, NIEHS/NIH, see page 68).

NIOSH/CDC is examining the relationship between female worker exposure to polychlorinated biphenyls and breast cancer. These compounds are suspected carcinogens because of their estrogenic and lipophilic properties (Whelan, NIOSH/CDC, see page 69).

Occupational Mixtures and Exposures

The NTP is coordinating an effort between NIEHS/NIH and NIOSH/CDC to better characterize worker exposures, educate workers, and identify occupational health research gaps. NIOSH/CDC is working with the NTP in nominating agents for study and designing laboratory studies and is undertaking its own research projects under this agreement. Current efforts are addressing worker exposure to asphalt fumes and 1-bromopropane and future initiatives are proposed for occupational mixtures such as welding fumes, abrasive blasting compounds, and metal working fluids (Morgan, NIEHS/NIH; Toraason, NIOSH/CDC).

Asphalt fumes generated during road paving have been linked to acute irritation of mucous membranes and skin, but to date no cancer risk has been established. Using a system designed to produce asphalt fumes similar to those found in the field, NIOSH/CDC has developed methods for characterizing these fumes and for monitoring asphalt fume exposure in inhalation toxicity studies. A study is underway to characterize asphalt fume composition including polycyclic aromatic hydrocarbons, develop a bioanalytical method that can be used to characterize exposure in laboratory inhalation studies, and assay gene expression response to asphalt fume exposure (Wang, NIOSH/CDC, see page 71). Laboratory inhalation studies are underway to evaluate the effects of exposure to asphalt fumes in cells and rodents (Ma, NIOSH/CDC, see page 48; Munson, NIOSH/CDC, see page 35).

An industry consortium has petitioned the EPA to list 1-bromopropane as an alternative for ozone-depleting solvents for general metals, precision, and electronics cleaning, aerosols, and adhesives. If this occurs, there is the potential for a vast increase in the exposure of workers and the public to this compound. Currently an appropriate occupational exposure limit for 1-bromopropane is not available from NIOSH/CDC, OSHA, or the American Conference of Governmental Industrial Hygienists. To obtain information on exposures to this chemical, NIOSH/CDC is conducting an industry-wide exposure assessment. The target population is a variety of industrial sectors: chemical, aerosol, and adhesive manufacturers; adhesive users; and the metal degreasing and electronics industries. Study sites will be selected on the basis of quantity and manner of 1-bromopropane use, number of workers exposed, type of manufacturing process, and representativeness of the industry. Exposure will be characterized using inhalation, exhaled breath, and biological measures. Results from this study will be used to 1) evaluate patterns of exposure, 2) develop and validate biomonitoring methods, 3) facilitate development of intervention recommendations for reducing exposures by engineering controls and work practice interventions, 4) develop occupational exposure limits, and 5) evaluate the suitability of 1-bromopropane as an alternative for ozone depleting solvents by the EPA (Hanley, NIOSH/CDC).

Following identification of major sites for occupational exposure to 1-bromopropane, the NIOSH/CDC will undertake a multifaceted health and exposure evaluation of those workers. End points to be assessed include biomarkers of internal dose, evaluations of reproductive dysfunction (both genders), neurotoxicity, genotoxicity, liver and kidney toxicity, and effects of exposure on hematologic end points. Biomarkers of exposure to both the parent compound and metabolites will be quantified along with workplace sampling. Data from this study may provide a basis for development of an occupational exposure limit for 1-bromopropane (Lynch, NIOSH/CDC).

The CERHR (see page 41) will hold an expert panel meeting in December 2001 to evaluate the evidence for potential reproductive and developmental toxicity resulting from exposure to 1-bromopropane and 2-bromopropane.

The NIOSH/CDC is undertaking a project to identify cohorts of workers exposed to reproductive toxicants and describe the demographic profiles of workers and companies. Data being collected include the quantity of chemical in use, the manner of use, the number of workers exposed, types of manufacturing processes, and any company data on reported morbidity or health concerns. Chemicals initially targeted are acrylamide, hydroxymethyl and methylene bis-acrylamide congeners, bisphenol A, and tricresyl phosphate. In-depth evaluations of reproductive function are planned for exposed workers (Moorman, NIOSH/CDC).

DNA-Based Products

DNA-based therapies are being developed for the treatment of a wide range of human diseases. However, by their very nature they pose a risk of interacting with the host genome or disrupting normal cellular processes in unexpected and unpredictable ways with potentially adverse consequences. Examples of DNA-based products include plasmid DNA encoding one or more antigenic proteins for vaccines against viral and bacterial pathogens, triplex forming synthetic oligonucleotides to modulate gene expression, and viral vectors for gene therapy. The FDA has only limited authority to require evaluation of non-acute, long-term safety risk associated with these therapies. In addition, the majority of the manufacturers of DNA-based products are small biotechnology companies and academic sponsors that lack the resources to perform long-term, large-scale studies on their products. Presently the NTP is collaborating with the FDA and sister NIH institutes to study the safety of DNA-based products and to address life-long risks presented by their use, the potential for reproductive toxicities and transmission of altered genetic material to subsequent generations, and the potential for DNA-based products to cause autoimmune disease or immune dysfunction (Irwin, NIEHS/NIH).

GENERAL TOXICOLOGY

Current Research Initiatives

Pre-Chronic Phase of Study

Studies in the pre-chronic phase are carried out usually through contract mechanisms at several U.S. laboratories and involve exposures of rats and mice of both sexes for periods of 14 to 90 days usually to chemicals, but sometimes to physical agents. Table 9 lists the agents currently in the pre-chronic phase in FY 2002; some of the studies target water disinfection by-products (see page 18) and herbal medicines (see page 19). Studies for 14 chemicals are ongoing from FY 2001 and studies for 14 chemicals are planned to start in FY 2002.

Table 9. Compounds in the Pre-Chronic Phase of NTP Study

Chemical Name	CAS No.	Project Leader[1]	Contract Project Officer[2]	Species: Strain	Route	Special Studies	Study Length[3]
Studies ongoing in FY2001 as of 10/09/01							
Androstenedione	63-05-8	Eastin	Chhabra/ Vallant	Mice: B6C3F1 Rats: Fischer 344	Gavage		14 days; 90 days
				Mice: B6C3F1 Rats: Fischer 344	Topical		14 days; 90 days
Bis(2-Chloroethoxy)methane	111-91-1	Dunnick	Orzech	Mice: B6C3F1 Rats: Fischer 344	Topical		14 days; 90 days
Bromodichloromethane (Water DBP)	75-27-4	Boorman	Chhabra/Vallant	Mice: B6C3F1 Rats: Fischer 344	Water		28 days
				Mice: B6C3F1 Rats: Fischer 344	Gavage		28 days
o-Chloropyridine	109-09-4	Chhabra	Chhabra/ Vallant	Mice: B6C3F1 Rats: Fischer 344	Topical		14 days; 90 days
Dibromoacetic acid (Water DBP)	631-64-1	Boorman	Chhabra/Vallant	Mice: B6C3F1 Rats: Fischer 344	Water		28 days
Estragole	140-69-0	Abdo	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage	Rats: P450s, serum gastrin	90 days
Goldenseal (powdered root)	GOLDENSEA LRT	Dunnick	Chhabra/Vallant	Mice: B6C3F1 Rats: Fischer 344	Feed		14 days; 90 days
Hexachlorobenzene	118-74-1			Rats: Sprague-Dawley	Gavage		
Isoeugenol	97-54-1	Abdo	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage	Rats: P450s, serum gastrin	90 days
β-Myrcene	123-35-3	Chan	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage	Rats: α2μ-globulin (optional)	90 days
Pulegone (herbal)	89-82-7	Chan	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage	Rats & mice: P450s; Glutathione-S-transferase	14 days; 90 days
Sodium thioglycolate	367-51-1	Hooth	Chhabra/ Vallant	Mice: B6C3F1 Rats: Fischer 344	Topical		14 days; 90 days
α-Thujone (herbal)	546-80-5	Hooth	Chhabra/ Vallant	Mice: B6C3F1 Rats: Fischer 344	Gavage		14 days; 90 days
α/β-Thujone mixture (herbal)	THUJONEMI XAB	Hooth	Chhabra/ Vallant	Mice: B6C3F1 Rats: Fischer 344	Gavage		14 days; 90 days
Studies proposed to start in FY2002 as of 10/09/01							
Acetaminophen	103-92-2		Chhabra/Vallant	Mice: B6C3F1 Rats: Fischer 344	Gavage	Gene expression profiles under daylight and night time lighting	48 hours
1-Bromopropane	106-94-5	Morgan	Orzech	Mice: B6C3F1 Rats: Fischer 344	Inhalation		14 days
Chromium picolinate monohydrate (CrIII)	27882-76-4	Abdo	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Feed	(optional) hydroxydeoxyguanosine, Glutathione-S-transferase	90 days

Chemical Name	CAS No.	Project Leader[1]	Contract Project Officer[2]	Species: Strain	Route	Special Studies	Study Length[3]
Cobalt metal	7440-48-4	Roycroft	Orzech	Mice: B6C3F1 Rats: Fischer 344	Inhalation		14 days
Diethylamine	109-89-7	Morgan	Orzech	Mice: B6C3F1 Rats: Fischer 344	Inhalation		14 days
Ginseng	50647-08-0	Chan	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage		14 days
1,2,3,4,6,7-Hexachloronaphthalene PCN66	103426-96-6	Hooth	Chhabra/ Vallant	Rats: Harlan Sprague Dawley	Gavage	Determine 1/2 life and relative potency based upon biochemical and toxic end points (P450s)	14 days; 90 days
1,2,3,4,6,7-Hexachloronaphthalene PCN67	103426-96-6	Hooth	Chhabra/ Vallant	Rats: Harlan Sprague Dawley	Gavage	Determine 1/2 life and relative potency based upon biochemical and toxic end points (P450s)	14 days; 90 days
Kava kava extract	9000-38-8	Chan	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage		14 days
Melatonin	73-71-4	Boorman	Chhabra/ Vallant	Mice: B6C3F1 Rats: Fischer 344	Gavage	3 different lighting conditions and special retinal exams; determine t1/2	14 days; 90 days
Methyl-*trans*-styryl ketone	1896-62-4	Cunningham	Chhabra/ Vallant	Mice: B6C3F1 Rats: Fischer 344	Topical		90 days
				Mice: B6C3F1 Rats: Fischer 344	Feed		
Milk thistle extract	84604-20-6	Dunnick	Orzech	Mice: B6C3F1 Rats: Fischer 344	Feed		90 days
Sodium dichromate dihyrate (CrVI)	7789-12-0	Abdo	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Water		90 days
Styrene-acrylonitrile trimer (SAN-Trimer)	SANTRIMER2	Chhabra	Orzech	Mice: B6C3F1 Rats: Fischer 344	Feed		7 weeks

[1] Project Leader - NIEHS/NIH staff scientist (unless otherwise indicated) who oversees each chemical's evaluation

[2] Contract Project Officer - NIEHS/NIH staff scientist (unless otherwise indicated) who coordinates research activities with the contract laboratory

[3] Study Length:

14 days and 17 days: Repeated dose study generally 14 or 17 days of exposure to be used for determining the dose range for the subchronic study. The doses (usually five doses plus control) tested cover a wide dose range; 5 animals/group, 2 genders, 2 species; complete necropies on all animals. Standard measurements: Organ weights (liver, thymus, right kidney, right testicle, heart, and lungs) are taken at necropsy on all animals surviving at the end of the study. Histopathologic evaluations are done only on those organ/tissues showing gross evidence of treatment-related lesions plus corresponding control animals.

90 days: Subchronic toxicity study generally 90 days of exposure to determine the toxic effects of the test chemical and to estimate the high dose for each gender of each strain and species to be tested in a chronic toxicity study. Five doses (plus control) are selected from the repeated dose study or based on other information; 10 animals/group, 2 genders, 2 species; complete necropies on all animals. Standard measurements: Organ weights (liver, thymus, right kidney, right testicle, heart, and lungs) are taken at necropsy on all animals surviving at the end of the study. Gross lesions are examined in all animals in all dose groups plus controls. Complete histopathologic evaluation is done on all control animals, all animals in highest dose group with at least 60% survivors at time of sacrifice, plus all animals in higher doses. Chemical-related lesions are identified and those organs plus gross lesions are examined in all lower doses to a no-effect level. A complete histopathologic evaluation is performed on all natural death/moribund sacrifice animals. Toxicological parameters evaluated include hematology, clinical chemistry, micronuclei determinations, and SMVCE (Sperm Morphology and Vaginal Cytology Evaluation): male organ toxicity is estimated by sperm motility, sperm count, and testicular spermatid head counts; female organ toxicity is evaluated by vaginal cytology and timing of the estrous cycle.

Mechanism-Based Toxicology

Chemical Disposition, Metabolism, and Toxicokinetics

Mechanistic information is obtained through evaluations of chemical disposition and metabolism. Those chemicals being evaluated in FY 2002 are listed in Table 10. Most studies are conducted in intact laboratory animals; some require incubations of human and rodent liver slices with the chemical. This information provides dosimetric data that can be integrated with other anatomical, biochemical, and physiological information into development of physiologically based pharmacokinetic/toxicokinetic models (see page 73). Such models are used increasingly in risk assessment to extrapolate between species, across dose ranges, and across different routes of exposure.

Table 10. Chemical Disposition, Metabolism, and Toxicokinetic Studies

Chemical Name	CAS No.	Project Leader[1]	Test[2]
Studies ongoing in FY 2001 as of 10/01/01			
Acelsulfame potassium	55589-62-3	Collins	Toxicokinetics
Allyl Acetate	591-87-7	Cunningham	Metabolism
Androstenedione	63-05-8	Cunningham	Chemical disposition
Bromodichloromethane	75-27-4	Smith	Toxicokinetics
2-Butyne-1,4-diol	110-65-6	Burka	Chemical disposition
Bis(2-Chloroethyoxy)methane	75-27-4	Smith	Toxicokinetics
o-Chloropyridine	109-09-1	Burka	Chemical disposition
Chromium picolinate monohydrate	27882-76-4	Burka	Chemical disposition
Dibromoacetonitrile	3252-43-5	Burka	Chemical disposition
Estragole	140-67-0	Cunningham	Chemical disposition
Hexachlorobenzene	118-74-1	Overstreet	Toxicokinetic
Methylene blue trihydrate	7220-79-3	Collins	Toxicokinetics
Myristicin	607-91-0	Cunningham	Metabolism
3,3,4,4,5-Pentachlorobiphenyl	57465-28-8	Smith	Toxicokinetics
2,3,4,7,8-Pentachlorodibenzofuran	57117-31-4	Smith	Toxicokinetics
Pulegone (Herbal)	89-82-7	Burka	Chemical disposition
Pyrogallol	87-66-1	Overstreet	Toxicokinetics
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	1746-01-6	Collins	Toxicokinetics

[1] Project Leader - NIEHS/NIH staff scientist who oversees each chemical's evaluation

[2] Test

Chemical disposition: determines fate of radiolabeled chemical, its absorption, distribution, metabolism and excretion.
Metabolism: studies fate of chemical, usually done *in vitro.*
Toxicokinetics: finding the concentration of a single chemical or its metabolites in blood or other biological tissues.

Drug Metabolizing Enzymes

A major objective of the NTP is to identify and characterize the acute and chronic toxicity of chemicals that humans encounter in their environment, work place, or as a result of food or drug consumption. Genetic polymorphisms in drug metabolizing enzymes affect the half-life, efficacy, and toxicity of clinically used drugs and influence susceptibility to environmentally caused diseases. Human cytochrome P450 enzymes (CYPs) metabolize foreign compounds, including clinically used drugs, carcinogens, and other xenobiotics, and genetic polymorphisms in these enzymes appear to account for variability among humans in metabolism of xenobiotics. The NIEHS/NIH is evaluating the reasons for drug and xenobiotic specificity of the CYP enzymes in humans. The CYP2C subfamily is highly homologous at the gene level, but differs markedly in their substrate specificity. Structure-activity studies and modeling are underway to identify the active site of human P450s. This information will enable prediction about how drugs or environmental chemicals may affect enzymatic activity and explain the effects of naturally occurring genetic polymorphisms. This group is also investigating what receptors are involved in the regulation of human CYP2Cs

(Goldstein, NIEHS/NIH). Researchers at the NIEHS/NIH and NCTR/FDA are working to identify new human CYP enzymes and any genetic polymorphisms, to develop genetic tests for these polymorphisms, to determine the amino acids important in substrate specificity of human CYP2Cs, and to identify the endogenous functional roles of CYP2C enzymes (Goldstein, NIEHS/NIH; Kadlubar, NCTR/FDA).

The NIEHS/NIH is characterizing the genotype/phenotype relationships in human cytochrome P450 enzymes in order to provide information critical for understanding differences in the therapeutic responses to many drugs, as well as human risk differences in susceptibility to environmental pollutants. These studies are investigating the metabolic activity of human recombinant cytochrome P450 enzymes in the CYP2C subfamily (Ghanayem, NIEHS/NIH). The NIEHS/NIH is studying the role of CYP2C enzymes in metabolism of environmental chemicals. An initiative focusing on the role of the cytochrome P450, CYP2E1 (thought responsible for oxidative metabolism of ethanol), in the toxicity of NTP chemicals has begun. Chemicals being studied include acrylonitrile and acrylamide (Ghanayem, NIEHS/NIH).

In addition to drugs, cytochrome P450 enzymes metabolize fatty acids to biologically active mediators that can have potent effects on vascular tone, ion transport, and peptide hormone secretion. Studies are underway at the NIEHS/NIH to identify and characterize new P450 isoforms that metabolize arachidonic acid to epoxyeicosatrienoic acids and hydroxyeicosatetraenoic acids and to study their regulation and functional significance. This work should provide insight into basic pathogenic mechanisms of selected diseases including ischemic heart disease, hypertension, and atherosclerosis (Goldstein/Zeldin, NIEHS/NIH).

Oxidative Stress

Understanding the mechanisms by which environmental toxicants act may enable early interventions in disease and allow preventive measures to be taken. Many human diseases are associated with reactive oxygen including cancer, heart disease, and neurodegenerative diseases. Mammalian peroxidases have a role in the metabolism of drugs and toxic chemicals. One NIEHS/NIH study is investigating the role of mammalian peroxidases in oxidative stress (Mason, NIEHS/NIH).

Researchers at the NCTR/FDA are also studying the consequences of mitochondrial DNA damage and its role in reactive oxygen-induced toxicity. The mitochondrion represents a target of reactive oxygen stress and mitochondrial DNA damage appears to be an early and sensitive marker of this stress. Hydrogen peroxide is produced through incomplete reduction of oxygen during oxidative phosphorylation and under certain conditions, such as inflammation, excessive amounts are produced. The impact of this excess hydrogen peroxide on subsequent adverse cellular events (*e.g.*, DNA damage, lipid peroxidation, glutathione depletion) is being addressed (Slikker, NCTR/FDA).

Free radical metabolites are possibly involved in the toxic effects of many drugs and environmental chemicals. Chemical reactions of free radical metabolites have known involvement in biochemical and toxicological consequences that cause cellular damage and death. Investigators at the NIEHS/NIH are using electron spin resonance and spin trapping to detect and identify free radical metabolites of toxic chemicals, drugs, and biochemicals and identify the role(s) and define the mechanisms associated with their radical-mediated toxicity (Mason, NIEHS/NIH). In addition, they are taking part in a multi-institutional initiative aimed at determining measurable, sensitive, non-invasive, and specific biomarkers for oxidative damage in animal models and humans. More than 25 putative markers measuring oxidative damage to lipids, proteins, DNA, and antioxidants have been evaluated using a rodent model (carbon tetrachloride poisoning). Several show significant promise – malonaldehyde and isoprostanes in plasma and urinary concentrations of isoprostanes (Mason, NIEHSNIH). The mass spectrometry lab at the NIEHS/NIH is developing an NICI/mass spectrometry (MS) isoprostane assay (Tomer, NIEHS/NIH). A spin trapping database (containing extensive biographic information) has been implemented at the

NIEHS/NIH and is accessible to both extramural and intramural scientists via the Internet (http://epr.niehs.nih.gov/stdb1.html) (Chignell, NIEHS/NIH).

Free radicals are thought to possibly play a role in the skin tumor promotion associated with occupational exposure to organic peroxides. A study is underway at the NIOSH/CDC to evaluate free radical generation in lipid extracts of mouse skin after exposure *in vivo* to CuOOH employing electron spin resonance and spin trapping. This study should help to clarify mechanisms of toxicity associated with redox intermediates in skin (Kommineni/ Shvedova, NIOSH/CDC).

Xenobiotic Transport Mechanisms

Research at the NIEHS/NIH is focusing on understanding the basic cellular mechanisms that drive drug and toxicant transport in specialized excretory (kidney, liver, and choroid plexus) and barrier tissues (brain capillary endothelium) and how these processes interact to determine chemical toxicity and drug efficacy. The vertebrate renal proximal tubule excretes a large number of potentially toxic chemicals through multiple, specific, transport proteins that remove them from the blood and concentrate them in urine. Studies are underway to define the extracellular signals and intracellular pathways that are involved in renal xenobiotic transport, and as possible, will be extended to identifying specific xenobiotic transport mechanisms in the brain capillaries and choroid plexus. This group is also focusing on development of imaging based techniques to define mechanisms responsible for transport of foreign chemicals out of the central nervous system. Recent findings include confirmation of the involvement of the transport protein OAT1 in uptake of organic anions from cerebrospinal fluid and investigation of how organic anions are transported from the choroids plexus to blood (Miller, NIEHS/NIH). Other studies at the NIEHS/NIH are examining the role of epithelial membrane transport in the elimination and toxicity of foreign chemicals and are applying that knowledge to study renal handling of toxic ions including herbicides and phenolphthalein or complexes (mercury-sulhydryl complexes) and its effects on toxicity. These transport systems are thought to play a criticial role in determining the persistence of foreign chemicals and drugs within the body (Pritchard, NIEHS/NIH). Other related studies are targeting the role of epithelial membrane transport in the elimination and toxicity of foreign chemicals from specialized compartments within the body. Current research is attempting to investigate sites that control the local concentration of toxicants within the brains, eye, and testis where the mechanisms responsible for removal of potentially toxic xenobiotics, drugs, and even neurotransmitter metabolites are largely unknown (Pritchard, NIEHS/NIH).

Atherosclerosis

Atherosclerosis is a leading cause of morbidity and mortality in the United States. Efforts continue for identifying agents that increase risk for this multi-factorial disease. A study at the NIEHS/NIH is examining whether environmental pollutants, such as carbon disulfide, might interact with other known risk factors, such as dietary fat, to exacerbate disease. An inhalation study is investigating the progression and mechanism of carbon disulfide atheroslcerosis in C57Bl/6 mice. Future studies will focus on apoE knockout mice to evaluate environmental exposures and atherosclerosis (Sills, NIEHS/NIH).

Electric and Magnetic Fields

Physical as well as chemical agents are of interest to the NTP. Under a Congressionally mandated program, the NIEHS/NIH in conjunction with the Department of Energy led an effort to determine what health effects, if any, arise from exposure to power-line frequency electric and magnetic fields (EMF) (Boorman/Portier/Wolfe, NIEHS/NIH). The NIEHS/NIH is currently working on updating a 1995 booklet for the public that provides basic information about EMF, summarizes research findings, to date, regarding possible health effects, and provides findings from recent reviews by expert scientific panels (Boorman/Wolfe, NIEHS/NIH). The NIEHS/NIH is also providing support for the International EMF Program sponsored by the World Health Organization and a manuel that focuses on management and communication of issues related to EMF (Portier/Wolfe, NIEHS/NIH).

Research on this topic is underway at the NIOSH/CDC to try and reproduce effects reported in the literature and to determine what exposure metric(s) is effective in causing a biological response that might lead to an adverse health effect. In addition, the study of potential biological effects from exposure to radiofrequency radiation associated with wireless communications is being included. The project is evaluating cellular responses to EMF, measuring cellular transformation, communication, and growth to clarify EMF effects, evaluate possible biomarkers of exposure, and identify the more biologically important exposure parameters. Studies focusing on radiofrequency radiation exposure effects in cultured brain cells are in progress (Savage, NIOSH/CDC). The NIEHS/NIH also has an initiative on radiofrequency radiation emissions underway (see page 17).

Toxicogenomics

National Center for Toxicogenomics

The NTP continually explores the use of new and improved test systems for improving its ability to evaluate potential toxicants. With the advent of novel molecular technologies, the NTP is moving into the arena of toxicogenomics – technology to apply the knowledge of genetics to the field of environmental medicine by studying the effect of toxicants on gene activity and the production of specific proteins by genes in response to toxicants. To oversee this effort and coordinate partners in academia, industry, and the public, the NIEHS/NIH created the National Center for Toxicogenomics (NCT) in 2000. The NCT will study the effect of toxicants on thousands of genes and the production of specific proteins by these genes in response to toxicants (Tennant, NIEHS/NIH). Genomic technologies, which are helping to move this field forward, include cDNA microarray, real time and quantitative polymerase chain reaction (PCR), and laser capture microdissection. The NIEHS/NIH and the NCTR/FDA are working on development of statistical methods for evaluating data from genomic technologies (Portier/Weinberg, NIEHS/NIH; Kodell, NCTR/FDA).

cDNA Microarray Technology

cDNA microarray technology has emerged as a gene expression tool by which scientists can detect genome-wide differential expression of thousands of genes and offers a methodological advancement for environmental health research. The application of a large number of genes or expressed sequence tags in a condensed array on a glass slide comprises a cDNA microarray. The NIEHS/NIH has developed a 2K and 12K human chip, 7K rat chip, 9K mouse chip, whole genome yeast chip, and a 1K *Xenopus* chip. This group has validated a “toxicogenomic strategy” for compound classification and prediction of unknowns and begun development of a database for storing microarray data for in-house purposes. The NTP hopes to use microarray technology to understand how environmental agents may affect gene expression thus elucidating their mechanisms of action (Afshari/Paules, NIEHS/NIH).

Real Time and Quantitative PCR (RTAQ-PCR) Technology

Quantitative RTAQ-PCR is used to measure specific levels of gene products within a sample obtained from either cells or tissues. Real-time, fluorescence detection of PCR products is a recent advancement in this technology that allows for quantitative analyses to be conducted more rapidly and on more samples at a time, thereby making this method favorable for large studies on gene expression and for the validation of genes identified in parallel gene expression profiling (microarray) studies. Efforts are ongoing at the NIEHS/NIH and NTP to use RTAQ-PCR for the analysis of inducible gene expression following exposure to a wide variety of compounds including dioxins, peroxisome proliferators, DNA damaging compounds, and immunotoxicants (Walker, NIEHS/NIH).

Laser Capture Microdissection Technology

A well established and highly utilized laser capture microdissection (LCM) laboratory is in place at the NIEHS/NIH. LCM enables accurate procurement of groups of specific cells or single cells of interest for molecular analysis. This technology can be used to microdissect pre-invasive lesions with subsequent molecular analysis in order to provide a genetic "fingerprint" of early changes and can be performed on stained (routine and immunohistochemical) or unstained, frozen or fixed specimens. In addition to DNA and RNA analysis of microdissected samples, the analysis of protein is being investigated. This technique will offer the NTP the ability to expand its use of molecular biology tools to characterize interactions of chemicals with critical target genes (Maronpot, NIEHS/NIH).

Areas for Future Initiatives and Resources

Development and Application of Genomic Technologies

The NTP continues to focus resources on the development of genomic technologies in order to be able to study more than a limited number of the chemicals, substances, or exposure circumstances to which individuals are exposed environmentally and occupationally. These technologies have potential for multiple applications: identification of toxicities for individual substances or mixtures, determination of dose-response relationships, identification of susceptible tissues and cell types, identification of expression patterns for specific cellular signals and processes at the molecular level, and cross-species extrapolation.

Both cDNA microarray and RTAQ-PCR have potential applicability for laboratory and epidemiology studies that would allow inclusion of mechanism-based end points by permitting the evaluation of potential molecular biomarkers of exposure and/or response. cDNA microarray technology could be used to characterize exposures and screen potential toxicants through direct comparison of the expression of thousands of genes simultaneously in control and exposed samples. Such arrays could be constructed targeting populations-at-large or to simulate populations-at-risk. By comparing gene expression patterns from various exposures, one could potentially identify "signature" patterns that might serve as biomarkers of potential exposure or potential disease. This would afford the opportunity to determine which gene expression patterns are most likely linked to environmental causes of human disease and greatly enhance initiatives such as an NIEHS/NIH project identifying genes responsive to environmental toxicants (see page 65) as well as serve as screening tools for identifying potential occupational and environmental toxicants. In addition, RTAQ-PCR affords a high throughput method for the analysis of DNA polymorphisms.

IMMUNOTOXICOLOGY

Current Research Initiatives

NTP immunotoxicity studies address adverse effects on the immune system that may result from occupational, accidental, or therapeutic exposure to environmental chemical, biological materials, or therapeutic agents. The identification of chemicals, which have potential to cause injury to the immune system, is of considerable public health significance as alterations in immune function can lead to increased incidence of hypersensitivity disorders, autoimmune or infectious disease, or neoplasias. Table 11 lists the substances under consideration for potential effects on the immune system. Testing for 16 chemicals is ongoing from FY 2001 and testing for 5 more is planned to begin in FY 2002. Several of these agents are water disinfection by-products and are being tested as apart of the Safe Drinking Water Program (see page 17). The NCTR/FDA is evaluating the effects of endocrine disrupting chemicals (see page 21) on the immune system in multigenerational studies. Patulin is a mycotoxin and children potentially have high exposure due to increased consumption of apple juice, a common vehicle. In response to contradictory findings about its potential immunosuppressive activity in mice, studies on Patulin were initiated in rats.

Table 11. Substances Being Tested for Immune System Toxicity[1]

Chemical	CAS No.	Species: Strain	Route	Testing Battery[2]
Studies ongoing in FY 2002 as of 10/09/01				
Cadmium Chloride	10108-64-2	Rats: Brown Norway	Gavage/Water	AI
Chloramine (Water DPB)	10599-90-3	Mice: B6C3F1	Water	IM
Chloroform (Water DPB)	67-66-3	Mice: B6C3F1	Water	IM
Dibromoacetic acid (Water DPB)	631-64-1	Mice: B6C3F1	Water	IM
Dichloroacetic acid (Water DPB)	79-43-6	Mice: B6C3F1	Water	IM
1,1-Dichloropropene	563-58-6	Mice: B6C3F1	Gavage	IM
Echinacea purpurea extract	90028-20-9	Mice: B6C3F1	Gavage	IM
Ethinyl estradiol (Endocrine disruptor)	57-63-6	Rats: Sprague-Dawley	*in utero*, Feed	IM, MG
Itraconazole	84625-61-6	Mice: B6C3F1	Gavage	RF
Patulin	149-29-1	Rats: Fischer 344	Gavage	IM
Pyrogallol	87-66-1	Mice: B6C3F1	Topical	HY
Rifamycin	14897-39-3	Mice: Balb/c	Topical	HY
Saquinavir mesylate (AIDS initiative)	149845-06-7	Mice: B6C3F1	Gavage	IM
Sodium chlorite	7758-19-2	Mice: B6C3F1	Water	IM
Trichloroethylene	79-01-6	Rats: Brown Norway	Gavage	AI
Vinclozolin (Endocrine disruptor)	50471-44-8	Rats: Sprague-Dawley	*in utero*, Feed	IM, MG
Studies proposed to start in FY 2002 as of 10/01/01				
1-Bromopropane	106-94-5			IM
Echinacea purpurea extract	90028-20-9			AI
Genistein	446-72-0			AI
Pyncogenol (grape seed and pine bark)				IM
Sodium dichromate dihydrate (CrVI)	7789-12-0			IM

[1] Project Leader - Dr. Germolec, NIEHS/NIH serves as the staff scientist who oversees each chemical's evaluation.
[2] Testing Battery
AI = Automimmunity studies
HY = Hypersensitivity, evaluated by the mouse ear swelling test and the local lymph node assay
IM = Immunomodulation, a two-tiered panel to evaluate the potential of agents to induce immunosuppression
MG = Multiple generations, assess effects on immunologic function and cancer end points for multiple generations
RF = Range finding, evaluation over a range of doses to identify a response

Dermal Hypersensitivity

A major area of research at the NIOSH/CDC focuses on dermal exposure including identifying potential occupational allergens, developing screening tools, understanding the mechanisms for dermal hypersensitivities, and evaluating intervention strategies. In an extension of previous work, a project is underway to evaluate chemical activation of innate immunity in allergic contact dermatitis. Using transgenic mouse models, this group has demonstrated that activation of innate immunity is required for development of allergic contact dermatitis. They will extend their work by focusing on the role of the innate immunity receptor, CD1, in development of this disorder and investigating the initial molecular and cellular events in skin that constitute toxicant activation of the cutaneous innate immune response (Tinkle, NIOSH/CDC).

Several NTP studies are focusing on natural latex rubber hypersensitivity. The route of latex exposure is believed to result in varied immunoreactive allergen profiles as defined by allergen-specific IgE recognition. One study is investigating the role of dextran glove powder in development of latex allergy by determining the mitogenic activity of dextran *in vitro* using murine and human models and evaluating the potential of naive dextran glove powder to elicit an IgE response in a rat inhalation model. An inhalation exposure system has been developed for exposing Brown Norway rats to latex proteins (Meade, NIOSH/CDC). Another study is focusing on understanding the importance of the routes of exposure (*e.g.*, dermal, inhalation, subcutaneous) in development of latex allergy by characterizing the allergen-specific IgE profile and levels in sensitized mice, determining the minimal natural rubber latex concentration necessary to elicit an IgE response, and identifying the primary allergen(s) responsible for causing the hypersensitivity. Additional studies are investigating the effects of co-exposure with other chemicals (*e.g.*, glutaraldehyde) or contaminants (*e.g.*, endotoxin) on development of an IgE-mediated response to latex proteins. This latter project is addressing the development of animal models of latex allergy and evaluating their utility. Such models could be useful for studying the mechanisms underlying latex allergy and for testing intervention strategies (Meade, NIOSH/CDCD).

The potential for latex proteins to penetrate into and through intact or abraded human skin is being evaluated *in vitro* using hairless guinea pig and human skin preparations. Sensitization of hairless guinea pigs via topical exposure is also being assessed *in vivo* as a model of natural rubber latex protein-induced Type I hypersensitivity. Future studies will focus on penetration of the individual latex proteins and on the effect of co-exposure to other occupational irritating and sensitizing chemicals (*e.g.*, glutaldehyde) for development of latex allergy (Meade, NIOSH/CDC).

NIOSH/CDC is working on improving the ability of mathematical/computer modeling to predict transdermal chemical penetration through human skin. *In vitro* penetration and diffusion studies are being performed using excised human cadaver skin. This information will be used to derive mathematical relationships that enable the prediction of systemic chemical exposures from realistic workplace dermal exposure scenarios (Frasch, NIOSH/CDC).

Contact dermatitis is a significant cause of occupationally induced morbidity and evidence is increasing that dermal chemical exposure may play a role in the induction of respiratory sensitization. A method of screening chemicals for their potential to induce irritation or IgE-mediated or T cell-mediated hypersensitivity responses is under development at the NIOSH/CDC. Such a method would be beneficial for identifying candidate chemicals and controlling their workplace levels or release into the environment. The method includes irritancy/phenotypic analysis using flow cytometric analysis. An elevation in B220+cells serves as an indicator of a potential sensitizer and an elevation in IgE+B220+cells as an indicator of the potential for induction of an IgE-mediated response. Gene array analysis is being conducted to identify novel genes that may be involved in chemically induced irritation, T-cell mediated, and IgE-mediated responses. Validation studies using a broad panel of chemicals and comparative analysis with other methods are ongoing (Meade, NIOSH/CDC).

Decontamination is important following contamination of a person's skin by a chemical. However, relatively little is known about factors affecting decontamination efficiency, such as properties of the contaminant, properties of the decontamination agent, delay time before decontamination, and skin health. A series of *in vitro* and *in vivo* studies are planned to address these issues using the hairless guinea pig (Soderholm, NIOSH/CDC).

Hepatotoxicity and Regeneration

An interest at the NIEHS/NIH is to identify the factors that can modify host resistance to endotoxin, such as hepatic damage or dysfunction, in order to characterize potential adverse interactions of toxic chemicals and bacterial products and to predict potential toxicity for humans. This is being addressed by focusing on *in vivo* mouse models of endotoxin hypersensitivity and the relationship between tumor necrosis factor (TNF)-α signaling and oxidative stress (Germolec, NIEHS/NIH).

Immune Cell Depletion and Host Resistence

The NIEHS/NIH in collaboration with FDA is studying the effect of monoclonal antibody-induced immune cell reduction on host resistance, as measured by flow cytometry. The goal is to examine the relationship between decrements in circulating immune cell phenotypes and susceptibility to infection or tumors. These results will be directly applicable to the interpretation of immune cell phenotype determinations in clinical medicine and for risk assessment in human populations exposed to potential toxicants. Initial dose-response and kinetic studies in C57B1/6 mice are underway. This study will also validate the usefulness of flow cytometry in non-clinical immunotoxicology studies (Germolec, NIEHS/NIH).

Granulomatous Lung Disease

This three part project will investigate the role of skin exposure, ultrafine particle exposure, and genetic susceptibility in the development of the pulmonary granulomatous disease, Chronic Beryllium Disease. The risk of Chronic Beryllium Disease has been associated with the HLA-DPB1 Glu69 gene and HLA-DPB1 transgenic mice have been developed for this study. 1) This project will investigate the interplay of cutaneous sensitization to beryllium with the development of beryllium-induced pulmonary granulomas in mouse models. 2) Backcrossing mice with different HLA haplotypes will be done to evaluate gene-environment interactions and the role of HLA homozygosity and heterozygosity in sensitization and disease. 3) Because exposure has been associated with ultrafine particles, the project will investigate the role of particle size and particle number on granuloma development. These studies will define the need for control of dermal exposures in work environments with elevated submicrometere particulate levels and assist OSHA in it re-evaluation of the beryllium worker exposure limit. This research will also advance our understanding of gene-environment interactions in the workplace (Tinkle, NIOSH/CDC).

Respiratory Disease

The respiratory system maintains an effective antimicrobial environment to prevent colonization by airborne microbes. Recent evidence suggests a role of neutrophils and natural killer (NK) cells in sustaining early stages of protective immunity. Researchers at NIOSH/CDC are studying the influence of airborne particulates on the function and dynamics of the macrophage-NK cell axis using an *in vivo* mouse model (Lewis, NIOSH/CDC).

The NIEHS/NIH is investigating the role of cyclooxygenases or prostaglandin H synthases (PGHS) in pulmonary response to environmental agents. This study is comparing pulmonary inflammatory indices of transgenic mice (PGHS-1 -/- or PGHS-2 -/-) to wildtype following allergen (ovalbumin) sensitization exposure. Efforts are also examining effects of disruption of Pghs genes on pulmonary responses to inhaled endotoxin (bacterial lipopolysaccharide) and exposure to vanadium pentoxide. Future studies will focus on elucidation of PGHS-dependent mechanisms in lung immune and inflammatory responses and evaluate host resistance in PGHS-deficient mice (Zeldin, NIEHS/NIH).

Occupational allergies are increasingly recognized as an important hazard in certain work environments and may play a role in the etiology of many workplace-related diseases. One area of interest is the effect of exposure to asphalt fumes. NIOSH/CDC has been working to characterize and reproduce field conditions experimentally. Reproducibility of the asphalt fumes within the NIOSH/CDC inhalation facility (see page 47) has been validated and studies are targeting effects on the immune system with the goal of identifying the active chemical component (Munson, NIOSH/CDC).

Infants dying of Sudden Infant Death Syndrome (SIDS) often have a pre-existing upper respiratory infection prior to death. Researchers at the NIEHS/NIH in cooperation with Duke University have developed a dual infection model of upper respiratory tract infection and bacterial sepsis that mimics the pathology of SIDS in rodents at a similar stage of immunologic maturity as at risk human infants. Efforts are underway to establish the specific immunological events and soluble mediators associated with the critical infection period and evaluate the use of cytokines or other immune measures as biomarkers for SIDS risk. Preliminary findings suggest that developmental exposure to environmental agents may alter immune parameters later in life (Germolec, NIEHS/NIH).

Workshop – Assessment of the Allergic Potential of Genetically Modified Foods

Both the general public and scientific community have a growing concern regarding the potential toxicity of genetically modified (GM) foods. Of specific interest is the ability of GM proteins to elicit potentially harmful immunologic responses including hypersensitivity and/or autoimmunity. The lack of information on the potential toxicity of these products has created a considerable backlash against the producers and users of these crops. To address these issues, the NTP, along with the EPA and FDA sponsored a workshop December 10-12, 2001 in Research Triangle Park, NC. This workshop was originally scheduled for September 24-25, 2001. Participates included experts in food allergy, GM crops, and the regulatory aspects of these products, along with bench scientists and clinicians. The specific aims of this workshop were to examine the current state of knowledge in the area, identify the critical issues regarding these materials, and develop testing strategies to examine the toxicity of these compounds. A meeting report is in progress for publication in *Environmental Health Perspectives* (Germolec, NIEHS/NIH).

Areas for Future Initiatives and Resources

Studies of Specific Areas

Very little is known about the potential for chemicals to affect the immune system, as well as understanding the basic cellular mechanisms of such effects. Continued initiatives are needed that focus on expanding that database especially for areas of high public or regulatory concern (*e.g.*, drinking water contaminants, DNA-based products, natural products, and therapeutic drugs) to provide information for use in risk assessment and for the accurate estimation of safe levels of chemical exposures. As part of these efforts, the NTP will begin assessments of the validity of previous analyses as to the sensitivity and predictivity of specific immune function tests. The program will continue its studies of the relationship between impairment of immune function and the organism's ability to resist infections or kill tumor cells through the NIEHS/NIH-FDA collaboration (see page 34) examining the effect of monoclonal antibody-induced immune cell reduction on host resistance. Future efforts will be directed toward host resistance studies to determine the relationship between lowering particular immune cell phenotypes and susceptibility to particular infections or tumors. Studies are proposed targeting the effect of individual AIDS therapeutics and combination therapies on flow cytometric parameters and, in tandem, host resistance in the C57Bl/6 mouse.

The public is continually concerned about *in utero* and postnatal chemical exposures resulting in adverse health outcomes in children and adults. The NTP has been a leader in the evaluation of developmental effects on the immune system through laboratory studies of perinatal exposure to pesticides, HIV therapeutics, and endocrine disrupting compounds. The NTP will continue this effort with future initiatives targeted at perinatal exposure to environmental agents in experimental models of autoimmune disease. In addition, priority will be given to a comprehensive assessment of the functional and structural development of targeted tissues, such as the nervous and immune system, following prenatal and perinatal exposure to test agent(s). These laboratory studies could be used in the future for either "stand-alone" assessment of developmental effects during the childhood and early adulthood periods or as the first level of a tiered approach for evaluations of children's health. The NTP will address this issue, in part, through expansion of assessments for immunotoxicity in rodent bioassays.

Animal Model Development

Animal experimental studies of autoimmune phenomena have enhanced insights into the underlying biology and plausibility of environmental factors as causative agents. However, there are no validated and generally applicable, predictive animal models for evaluating the potential of environmental agents to induce or exacerbate autoimmune disease. Such models, in combination with current knowledge about human immune system biology, would facilitate the design of better-formulated studies for investigating disease etiology.

NEUROTOXICOLOGY

Behavioral and neurologic alterations in response to deleterious environmental agents often represent the earliest observable manifestation of toxicity. Neurotoxicology screening of NTP compounds often employs the EPA Neurobehavioral Screening Battery, Functional Observational Battery (FOB), with addition of locomotor activity measurements or the NIEHS Neurobehavioral Test Battery. The testing batteries examine the various neurobehavioral systems: sensory, motor, autonomic, and peripheral nervous system. The FOB employs observational screening while the NIEHS test battery uses automated test systems to evaluate the various nervous system components. Neurotoxicology testing is generally done in association with subchronic 90-day testing and Table 12 lists substances being evaluated for possible neurotoxicity. Eight studies are ongoing from FY 2001 and 3 are proposed to start in FY 2002. Carbonyl sulfide was nominated for NTP study because it is a high production hazardous air pollutant and health data is needed under the Toxic Substances Control Act. Neurobehavioral batteries are also being included in assessments of occupational cohorts (see Exposure Assessment, page 70).

Table 12. Substances Undergoing Neurotoxicity Assessment

Chemical	CAS No.	Project Leader[1]	Species: Strain	Route
Studies ongoing in FY 2002 as of 10/01/01				
Dibromoacetic Acid	631-64-1	Harry	Rats: Fischer 344	Water
Diethanolamine	111-42-2	Irwin	Rats: neonatal	Gavage
Ethinyl Estradiol (Endocrine disruptor)	57-63-6	Delclos (NCTR/FDA)	Rats: Sprague-Dawley	Feed
Methoxychlor (Endocrine disruptor)	72-43-5	Delclos (NCTR/FDA)	Rats: Sprague-Dawley	Feed
Molinate	2212-67-1	Harry	Rats: Fischer 344	Feed
6-Propyl-2-thiouracil	51-52-5	Harry	Rats: Long Evans Hooded	Water
Tellurium	13494-80-9	Harry	Rats: Long Evans Hooded	Feed
Vinclozolin (Endocrine disruptor)	50471-44-8	Delclos (NCTR/FDA)	Rats: Sprague Dawley	Feed
Studies proposed to start in FY 2002 as of 10/09/01				
Carbonyl Sulfide	463-58-1	Morgan	Rats: Fischer 344	Inhalation
Dibromoacetonitrile (Water DBP)	3252-43-5	Harry	Rats: Sprague-Dawley	Water
Metolachlor	51218-45-2	Harry	TBD[2]	TBD

[1] Project Leader - NIEHS/NIH staff scientist who oversees each chemical's evaluation, unless otherwise indicated.
[2] TBD: to be determined

Current Research Initiatives

Biomarkers of Neurotoxicity

Broadly applicable biomarkers of neurotoxicity, both for use in humans and animals, are not available for screening the thousands of potentially neurotoxic compounds or exposures found in the environment and workplace. Workers who use vibrating tools are at increased risk for developing hand-arm vibration syndrome (HAVS). Vasospasms in response to cold temperatures; numbness, parasthesia, and a loss of grip strength and changes in nerve and blood vessel morphology characterize HAVS. Studies at NIOSH/CDC are investigating HAVS etiology, including the biological pathways involved in its development and progression in order in the future to develop tools for screening (Lindsley, NIOSH/CDC).

Developmental Neurotoxicology

The developing nervous system can be vulnerable to the effects of chemical exposures that may cause subtle alterations in the brain's organization and lead to dysfunction in the mature organism in the absence of gross neuropathology or neurochemical changes. During development, gene expression is an active process and is required for neural and glial growth, development, and interactions. These critically time events are assumed to be important in the differential susceptibility of the developing organism to environmental insult. Various environmental agents (chemical and physical) are being tested for their ability to alter the spatio-temporal expression of mRNA for various developmentally regulated proteins. Specifically, chemicals which disrupt thyroid hormone levels are being examined to determine effects on neuronal- and myelin-related genes and development of the neural network. Additional studies will evaluate the role of interleukin-6 in development of the nervous system as well as its acute toxicity on early postnatal development of the central nervous system (Harry, NIEHS/NIH).

NCTR/FDA staff is interested in examining neuroanatomical and neurobehavioral end points associated with exposure to endocrine disrupting chemicals (see page 21). Within the area of excitatory amino acid/mediators of neuroanatomical susceptibility to neurotoxicants, NCTR/FDA scientists are studying neonatal hormonal conditions that can change adult reproductive behaviors and evaluating the biochemical events occurring during the apopotic and proliferative sexual differentiation stages of the developing and maturing brain (Scallet/Ferguson, NCTR/FDA).

NCTR/FDA scientists are working to develop and validate quantitative biomarkers and immediate precursor events of neurotoxicity and elucidate the modes of action of neurotoxicants. Unique features of these research efforts include the capability to determine target tissue concentrations and cellular interactions of suspected neurotoxicants. Focal areas of interest are 1) excitatory amino acids as mediators of development, aging and neuroanatomical susceptibility to neurotoxicants; 2) the role of aromatic monoamines in neurotoxicity; 3) disruptors of energy metabolism and axonal transport; 4) oxidative-stress-induced neurotoxicity; 5) interspecies extrapolation and validation of animal models; and 6) development, validation, and application of novel neurohistochemical tracers (Slikker, NCTR/FDA).

Neurodegeneration

Inflammatory processes and initiation of the cytokine cascade within the nervous system have been hypothesized as critical in the development of various neurodegenerative diseases. A cytokine response has been identified in neurodegenerative disorders targeting either neurons (Alzheimer’s Disease), axons (peripheral nerve axonopathy), or the myelin sheath (multiple sclerosis). The NIEHS/NIH is evaluating the role of specific cells, cytokines, and growth factors in the inflammatory response within the brain and their relevance to neurodegeneration. These studies may help determine whether there is a contributing role for an early pro-inflammatory response in subsequent degeneration. Preliminary data suggest that cell cycle regulation is critical to the stage-specific response of microglia cells. Future efforts will focus on whether the microglia’s response and its associated cytokine or toxic product formation and release are critical for astrocyte reactivity and neurodegeneration (Harry, NIEHS/NIH).

Studies at the NIEHS/NIH are focusing on the effect of chronic exposure to carbon disulfide and neurodegenerative disease. This effort includes comparative studies of the central nervous system between rats and mice. Carbonyl sulfide is the oxidation product of carbon disulfide. The NIEHS/NIH is also assessing the neurotoxicity of carbonyl sulfide in an inhalation study and this information will be used for planning longer term studies and evaluating mechanism(s) of toxicity (Sills, NIEHS/NIH).

Areas for Future Initiatives and Resources

Studies of Specific Areas

Neurodegenerative diseases such as Alzheimer’s Disease, Parkinson's Disease, and multiple sclerosis are proposed to have environmental origins. Continued NTP research is needed on the mechanisms related to how toxicant exposure may contribute to disease etiology and on developing models for studying neurotoxicity and susceptibility. Such information would greatly facilitate the development of therapeutic intervention approaches addressing these diseases.

Another area for future initiatives focuses on understanding the impact of exposure to environmental and workplace agents on the nervous system during maturation and development. The developing nervous system can be selectively vulnerable to insult by environmental and workplace toxicants resulting in long-term alterations in overall neurological function. Research would address the interdependency between processes such as learning and memory, repair and regeneration, and modulation of individual responses to stress induced by environmental agents, and as possible, use this information to identify biomarkers useful for neurotoxicity screening. The NTP will also address this issue, in part, through expansion of assessments for neurotoxicity in rodent bioassays.

PHOTOTOXICOLOGY

NTP Center for Phototoxicology

The NTP Center for Phototoxicology (NCP) was established in 2000 at the NCTR/FDA to conduct mechanism-based research and phototoxicology and photocarcinogenesis studies on substances nominated to the NTP. Many of these compounds are of regulatory interest to the FDA. Dr. Paul Howard, NCTR/FDA, serves as the Center Director.

The NCP's state-of-the-art laboratory can study the potential toxic or carcinogenic effects of a test substance in combination with electromagnetic radiation from several light sources. The NCP also conducts mechanistic studies to learn how these effects might occur. This laboratory is designed to allow study of many types of photoactive and environmental agents (*e.g.,* cosmetics, tanning enhances, drugs, herbals, etc.) for ultraviolet (UV) radiation- or simulated solar light-induced toxicity and cancers. Studies conducted in this facility should contribute to providing high quality data upon which to base public health decisions about the interactions of drugs or other compounds with sunlight. The key features of this facility are the two six-inch zenon arc lamps that operate at 6,500 watts. The visible and UV radiation emitted from each lamp, when filtered through glass designed to simulate the earth's atmosphere, closely mimics the spectrum of solar light. About 5,000 mice can be exposed per day to the simulated solar light making this facility unique for handling the large number of animals required for carcinogenicity studies. The facility also has a portable fluorescent lamp assembly that can be equipped with most of the available fluorescent lamps (*e.g.,* UV-B lamps, tanning lamps, and germicidal lamps) for use in studying the biological effects of these light sources on animals.

Expansion of the animal facility began in spring 2001. Upon completion of this expansion, the laboratory will have the capacity for the simultaneous photocarcinogenicity testing of up to four chemicals.

Chemicals are nominated for testing by each of the FDA centers and offices within the FDA Commissioner's Office. A FDA committee (Phototoxicology Chemical Selection Working Group) prioritizes the nominations and forwards them to the ICCEC for entry into the NTP nomination and selection process (see page 10). A standing committee (Toxicology Study Selection and Review Committee), composed of scientists with expertise in this area from FDA, NIEHS/NIH, other Federal agencies, and academia, reviews the design of protocols and progress on studies. Such studies should generate critically important scientific data for use in determining potential human health risks from the effects of therapeutic agents, chemicals used in cosmetics, device materials, food additives and supplements, tanning enhancers, *etc*., on light-induced skin toxicity and skin cancer. The NTP Board of Scientific Counselors (see page 2) advises the NCP on programs and priorities.

Contact information: NTP Center for Phototoxicology, Dr. Paul C. Howard, Director, NCTR/FDA, HFT-110, 3900 NCTR Road, Jefferson, Arkansas, 72079; T: (870) 543-7137; phoward@nctr.fda.gov. NCP web site: www.fda.gov/nctr/sciences/phototox.htm

Current Research Initiatives

Dermatological Chemoexfoliation

A study at the NCTR/FDA is investigating the effects of dermatological chemoexfoliation using α- and β-hydroxy acids on cell proliferation and DNA damage in the hairless mouse exposed to simulated solar light. For details see page 18 (Howard, NCTR/FDA).

Photosensitization

Photosensitization can result when light interacts with endogenous or exogenous chemical agents in the skin and eyes. This process can produce undesirable clinical consequences, such as exaggerated sunburn, allergic reactions, or skin cancer, or can have beneficial effects as in tumor photodynamic therapy or psoralen (PUVA) therapy for psoriasis. NIEHS/NIH scientists are studying the photochemical mechanisms whereby photosensitizers exert their toxic or therapeutic effects. Agents being examined include fluoroquinolones, a relatively new class of antibiotics useful in the treatment of gram-negative bacterial infections, to determine why this class of drugs is phototoxic and why related lomefloxacin and fleroxacin are photocarcinogenic. Other studies are investigating the herbals, berberine, found in Goldenseal (see page 19), xanthurenic acid, a component of cataracterous lenses, and in collaboration with scientists at North Carolina State University, the mechanism of *Cercosopera nicotianae* resistance to single oxygen generating phototoxins. Specifically they are evaluating the roles played by free radicals and other active oxygen species in this process with future objectives of being able to predict photosensitizing potential and developing intervention strategies (*e.g.*, sunscreens, systemic protection, eye drops) (Chignell, NIEHS/NIH).

UV-Induced Carcinogenesis

Researchers at NIOSH/CDC are studying the mechanisms of toxicity and carcinogenicity of UV radiation in skin diseases (Ding, NIOSH/CDC; see page 19). A project at the NIEHS/NIH is investigating the role(s) of cyclooxgenases 1 and 2 in UV-induced skin cancer (Langenbach, NIEHS/NIH, see page 62).

Phototoxicology Methods Development

In order to understand the photochemistry and photophysics of environmental chemicals it is necessary to use modern chemical analysis and spectroscopic techniques of many kinds. The following spectrometers are being built and tested at the NIEHS/NIH: steady state and lifetime spectrophotofluorometer, steady state and lifetime singlet oxygen luminescence spectrometers, and a laser flash photolysis spectrometer (Chignell, NIEHS/NIH).

Areas for Future Initiatives and Resources

Laboratory Expansion

Exposure of the U.S. population to UV radiation and the use of topical skin agents are increasing annually. The NCP offers a unique opportunity for the NTP to become a leader in the evaluation of these types of substances for their phototoxic and photocarcinogenic potentials. The laboratory has expanded its animal facility to handle simultaneous photocarcinogenicity testing of up to three chemicals. This expansion will enable testing of alternate animal models (*e.g.*, transgenic) for their suitability as replacements for the SKH-1 albino mouse and addressing phototoxic effects for end points other than cancer, such as photoimmunotoxicity and ocular toxicity.

Studies of Specific Diseases

The SKH-1 mice get cataracts when exposed to simulated sunlight. With expansion of the laboratory, the NTP plans to initiate studies to characterize this effect and investigate its etiologic mechanism.

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

NTP Center for the Evaluation of Risks to Human Reproduction

The NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) was established in 1998 to serves as an environmental health resource to the public and regulatory and health agencies. The CERHR is located at the NIEHS/NIH and Dr. Michael Shelby serves as the Center Director. It provides scientifically based, uniform assessments of the potential for adverse effects on reproduction and development caused by agents to which humans are exposed. The NTP Board of Scientific Counselors (see page 2) advises the Center on its processes, priorities, and direction.

The CERHR follows a formal process for nomination, selection, and review of chemicals that includes opportunity for public participation. The CERHR selects chemicals for review based on several factors, including production volume, extent of human exposures, public concern about the chemical hazard, and published evidence of reproductive or developmental toxicity. CERHR expert panel meeting are held in public forums.

The CERHR web site (http://cerhr.niehs.nih.gov) has information covering common questions and concerns regarding healthy pregnancy and the potential of various exposures to adversely affect the development of children.

Contact information: NTP Center for the Evaluation of Risks to Human Reproduction, Dr. Michael Shelby, Director, NIEHS/NIH, P.O. Box 12233 EC-32, 111 T.W. Alexander Dr., Research Triangle Park, NC 27709; T: (919) 541-3455; shelby@niehs.nih.gov. CERHR web site: http://cerhr.niehs.nih.gov

Expert Panel Peer Reviews

Methanol

The CERHR held an expert panel review of methanol (CAS No. 67-56-1) October 15-17, 2001, in Alexandria,Virginia. A large toxicity database exists on the reproductive and developmental effects of methanol. Methanol is a commercially important, high-production-volume chemical with potential for occupational, consumer and environmental exposure. Once finalized, the expert panel report will be posted on the CERHR web site and public comment solicited.

Bromopropanes

The CERHR held an expert panel review of 1-bromopropane and 2-bromopropane December 5-7, 2001, in Herndon,Virginia. 1-Bromopropane has various industrial uses and is being considered as a replacement for ozone-depleting chemicals such as hydrochlorofluorocarbons and chlorinated solvents. 2-Bromopropane has no industrial uses in the United States but is a contaminant in 1-bromopropane. Once completed, the expert panel report will be posted on the CERHR web site and public comment solicited.

Ethylene Glycol and Propylene Glycol

A future expert panel review of ethylene glycol and propylene glycol is planned. Ethylene glycol is a high-production-volume chemical used chiefly in antifreeze for heating and cooling systems. Propylene glycol is used as an antifreeze, de-icing solution, and in various paints and coatings and is approved for use in various food additives, drugs, and cosmetics.

NTP-CERHR Monographs

Following an expert panel meeting and receipt of public comments on the report, the CERHR prepares an NTP-CERHR monograph for transmittal to federal and state agencies, interested parties, and the public. This document contains an NTP brief giving the program's interpretation of the potential for the chemical to cause adverse reproductive and/or developmental effects for humans exposed to it. The CERHR is currently preparing the transmittal documents for seven phthalate esters.

Current Research Initiatives

The NTP has developed a wide range of techniques and testing regimes (Table 13, Column 6) for evaluating potential toxic effects of chemical exposure on the reproductive system of rodent models and/or the developing embryo of rodent models. A variety of agents are being evaluated by the NTP for their potential reproductive or developmental toxicity. The study of 27 compounds is on-going from FY 2001 and 8 studies are proposed to start in FY 2002. Some of these substances are identified as possible drinking water contaminants (see page 17) or endocrine disrupting agents (see page 21); others are herbal medicines (see page 19). Pesticides (*e.g.*, carbaryl, chlorpyrifos, heptachlor, methoxychlor, and tebuconazole) are under study for determining whether developmental exposures have lasting effects on functioning of the nervous, immune, or reproductive system in adult rats (Juvenile Pesticide Assessment; Bishop, NIEHS/NIH). Many of the *in vivo* and *in vitro* laboratory activities complement human reproductive and developmental initiatives in environmental epidemiology and exposure assessment (see pages 64 and 70, respectively).

Children's Health

The NTP continues to be a leader in issues related to children's health through research and the CERHR (see page 41). The Juvenile Pesticide Assessment (see Table 13) is evaluating issues related to children's health and functional deficiencies previously identified in human adults who received perinatal and/or prepubertal exposures to pesticides. In these evaluations, relatively high chemical exposure doses are being included in order to obtain information useful to regulatory agencies for making determinations about human safety indices and for comparing the data with currently acceptable levels of allowable pesticide residues in the food supply. The NTP has ongoing efforts to evaluate effects of various agents on developing immune and nervous systems through laboratory studies of pesticides, water disinfection by-products (DBPs), and endocrine-disrupting agents. The program is expanding these efforts by establishing study protocols where perinatal animals will be given these agents and then examined for developmental immunotoxicology, neurotoxicology, and reproductive effects. Toxicokinetic data from mothers, fetuses, and newborns will be used to develop physiologically based pharmacokinetic models of risk to humans from environmental toxicants during perinatal development (see page 73).

Table 13. Substances under Consideration for Reproductive and Developmental Toxicity

Chemical	CAS No.	Project Leader[1]	Species: Strain	Route	Testing Battery[2]
Studies ongoing in FY 2002 as of 10/09/01					
3'-Azido-3'-Deoxythymidine (AIDS initiative)	30516-87-1	Bishop	Mice: Swiss (CD-1)	Gavage	RACB
3'-Azido-3'-Deoxythymidine/ 2',3'-Dideoxycytidine (AIDS initiative)	AZTDDCCOMB	Jahnke	Mice: Swiss (CD-1)	Gavage	TER
3'-Azido-3'-Deoxythymidine + 2',3'-Dideoxyinosine (AIDS initiative)	AZTDDICOMB	Bishop	Mice: C57BL/6	Gavage	RACB
Benzophenone	119-61-9	Jahnke	Rats: Sprague-Dawley	Gavage	TER
			Rabbit: New Zealand White	Gavage	TER
					TRP
Berberine chloride dihydrate	5956-60-5	Jahnke	Mice: Swiss (CD-1)	Feed	TER
					TRP
			Rats: Sprague-Dawley	Feed	TRP
Carbaryl (Juvenile Pesticide Assessment)	63-25-2	Harris	Rats: Sprague-Dawley	Gavage	JPA
Chlorpyrifos (Dursban) (Pesticides in children)	2921-88-2	Harris	Rats: Sprague-Dawley	Gavage	JPA
Dibutyl phthalate	84-74-2	Bishop	Rats: Sprague-Dawley	Feed	RACB
Dibutyl phthalate/ Flutamide mixture	DPB/FLUTAMID	Bishop	Rats: Sprague-Dawley Rats: Wistar	Gavage	RACB
cis-Dichlorodiamine platinum	15663-27-1	Harris	Rats: Sprague-Dawley	IP/IJ[3]	TER
cis & *trans* 1,2-Dichloroethylene	540-59-0	Heindel	Rats: Sprague-Dawley	Gavage	TRP
Di(2-ethylhexyl) phthalate	117-81-7	Bishop	Rats: Sprague-Dawley	Feed	RACB
Emodin	518-82-1	Jahnke	Mice: Swiss CD-1	Feed	TER
			Rats: Sprague-Dawley	Feed	TER
					TRP
Ethinyl estradiol (Endocrine disruptor)	57-63-6	Delclos (NCTR)	Rats: Sprague-Dawley	Feed	MG
Genistein (Endocrine disruptor)	446-72-0	Delclos (NCTR)	Rats: Sprague-Dawley	Feed	MG
Goldenseal (powdered root) (Herbal)	GOLDENSEALRT	Jahnke	Rats: Sprague-Dawley	Feed	TER
			Mice: Swiss (CD-1)	Feed	TER
Heptachlor (Juvenile Pesticide Assessment)	76-44-8	Harris	Rats: Sprague-Dawley	Gavage	JPA
Hexachlorobenzene	118-74-1	Bishop	Rats: Sprague-Dawley	Gavage	RACB
Isoeugenol	97-54-1	Bishop	Rats: Sprague-Dawley	Gavage	RACB
		Hunter	Rabbit: New Zealand White	Gavage	TRP
Methoxychlor (Endocrine disruptor)	72-43-5	Delclos (NCTR)	Rats: Sprague-Dawley	Feed	MG
Methoxychlor (Juvenile Pesticide Assessment)	72-43-5	Harris	Rats: Sprague-Dawley	Gavage	JPA
Nonylphenol (Endocrine disruptor)	104-40-5	Delclos (NCTR)	Rats: Sprague-Dawley	Feed	MG
Silver acetate	563-63-3	Jahnke	Rats: Sprague-Dawley	Gavage	TRP
Sodium bromate (Water DPB)	7789-38-0	Bishop	Rats: Sprague-Dawley	Water	RACB
α-Solanine	20562-02-1	Jahnke	Mice: Swiss Albino	Gavage	TER
Tebuconazole	80443-41-0	TBD[4]	Rats: Sprague-Dawley	Gavage	JPA
Vinclozolin (Endocrine disruptor)	50471-44-8	Delclos (NCTR)	Rats: Sprague-Dawley	Feed	MG
			Mice: Swiss (CD-1)	Feed	TER
Studies proposed to start in FY 2002 as of 10/09/01					
Berberine chloride	5956-60-5	Jahnke	Rats: Sprague-Dawley	Gavage	TER

Chemical	CAS No.	Project Leader[1]	Species: Strain	Route	Testing Battery[2]
			Mice: Swiss (CD-1		
DBP mixture (Water DPB)	DWDBPMIXTURE	Bishop	Rats: Sprague-Dawley	Water	RDGT
2'3'-Dideoxyinosine + D4T (AIDS initiative)	DDI/D4TCOMB	Jahnke	Mice: Swiss (CD-1)	Gavage	TRP
Ethinyl estradiol (Prepubetal study)	57-63-6	Bishop	Rats: Sprague-Dawley	Gavage	RACB
Flutamide (Prepubetal study)	13311-84-7	Bishop	Rats: Sprague-Dawley	Gavage	RACB
Methoxychlor (Prepubetal study)	72-43-5	Bishop	Rats: Sprague-Dawley	Gavage	RACB
Silver acetate	563-63-3	Jahnke	Rats: Sprague-Dawley	Gavage	TER
Vinclozolin (Prepubetal study)	50471-44-8	Bishop	Rats: Sprague-Dawley	Gavage	RACB

[1] Project Leader - NIEHS/NIH staff scientist (unless otherwise indicated) who oversees each chemical's evaluation

[2] Testing Battery

JPA = Juvenile pesticide assessment; exposure during development with assessment of central nervous, immune, and reproductive systems in the adult animal.

MG = Multigenerational endocrine disruptor studies; assess effects on reproduction and cancer end points over multiple generations.

PZE = Preimplantation zygote effects; a test in mice used to examine effects during the earliest stages of embryo development (equivalent to the first trimester in humans) and to identify those effects that may cause birth defects or other developmental toxicities if the embryo is exposed during these stages of development.

RACB = Reproductive Assessment by Continuous Breeding; two-generation study design for identifying long-term effects on male and/or female reproduction, characterizing toxicity, and examining dose-response relationships of the test compound.

RDGT = Reproductive/developmental & general toxicity; identify the physiologic processes (development; female reproduction; male reproduction; various somatic organs/processes) that are the most sensitive to exposure.

TER = Teratology; examines both maternal factors of the pregnant dam as well as the health and well being of the developing fetus including body weight, visceral and skeletal examination, and as possible, LOAEL (lowest observed adverse effect level) and NOAEL (no observed adverse effect level) are determined for maternal and fetal toxicities.

TRC = Total reproductive capacity; detection of infertility, spontaneous abortion, fetal and neonatal death, birth defects, and genetic susceptibility.

TRP = Teratology pilot study; dose screening study designed to help set doses for the definitive teratology study. Study design considers maternal toxicity, fetal toxicity, fetal body weight, and external malformations, but does not include visceral or skeletal examination of the fetus.

[3] IP/IJ = Intraperitoneal Injection

[4] TBD = to be determined

Female Reproductive System

Researchers at the NCTR/FDA are actively involved in experiments designed to define the normal and estrogen-altered reproductive tract developmental profiles using the rodent model. This information is being used to create and validate a computerized knowledge base. Using this information several Quantitative Structural Activity Relationship (QSAR) models for chemical binding to estrogen receptors are under development (Anson, NCTR/FDA).

The NIEHS/NIH is evaluating the toxicity of carbonyl sulfide (see also page 38 and 46). A short-term study is planned to determine potential reproductive and developmental effects. A two-generation study is proposed to assess effects on lactation and reproductive performance of the second generation following exposure during development (Sills, NIEHS/NIH).

Male Reproductive System

Studies at the NIOSH/CDC are exploring how exposure to environmental/occupational chemicals that mimic or antagonize the effects of endogenous hormones (especially estrogens or androgens) can adversely affect normal developmental/reproductive functions regulated by these hormones (Murono, NIOSH/CDC, see page 22).

Through an interagency agreement with the Lawrence Livermore National Laboratory/Department of Energy, the NIEHS/NIH is sponsoring development of an assay that employs fluorescence *in situ* hybridization (FISH) to detect structural and numerical chromosome damage in sperm and early embryonic cells of rats and mice. Currently, FISH is being used to evaluate the aneugenic and clastogenic potential of environmental chemicals tested in NTP bioassays, as well as to address questions about dose response, differential stage sensitivity, and the relationship between defects found in sperm and those transmitted to the embryo. Efforts will continue to validate the use of the rodent assays (Bishop, NIEHS/NIH).

Mammalian Mutagenesis

Somatic and germ cell mutations can have a severe impact on the fitness of multicellular organisms and their offspring, respectively. The NIEHS/NIH has developed a procedure using the well-characterized *am3* mutation of PhiX 174 to differentiate mutations that are fixed within C57BL6/J mice from those that arise from *ex vivo* events that damage DNA. The NIEHS/NIH is continuing to test this procedure for 1) studying mutations induction by environmental toxicants during development, 2) investigating homeostasis of mutations during the aging process, 3) identifying characteristics of tumor progression from preneoplastic to neoplastic growth in relation to genomic stability, and 4) evaluating systemic effects of somatic mutations in development of age-related degenerative diseases (Malling, NIEHS/NIH).

Teratology

Field studies have confirmed that there is a significant elevation in the frequency of malformed amphibians at several study sites in Minnesota and Vermont. Studies are continuing at the NIEHS/NIH to identify the etiology of these effects. Water and sediment samples are being tested for the capacity to cause malformation of frogs in the laboratory. Studies continue about whether pathways associated with thyroid metabolism may be involved, and future efforts will target gene markers for frog embryogenesis, retinoid/thyroid receptor binding, endocrine response, and transgenic fish mutagenesis assays. It is proposed that a complex mixture of man-made chemical degradation products and natural compounds may be acting synergistically to cause these effects; this hypothesis is being tested (Burkhart, NIEHS/NIH).

Areas for Future Initiatives and Resources

Areas for Specific Studies

The NTP will continue its efforts to develop methods and strategies for identifying the effects of environmental and occupational toxicants on reproductive and developmental end points and recognizes that this area is one of increasing public interest. Future initiatives will address the unique sensitivities of developing humans to toxic insults and the potential for consequential exposure-related adverse health effects that may manifest themselves anytime from conception through adulthood. In addition, research efforts will be directed toward the sites and mechanisms of action of reproductive and developmental toxicants and on developing a wide range of techniques for evaluating those effects. To facilitate this research effort, the NTP is modifying the concept for toxicology and carcinogenesis studies that is used to employ contract mechanisms for these studies using animals. The expanded concept includes the potential for studying *in utero* and postnatal exposures and for expanding the range of assessments to include developmental immunotoxicity, neurotoxicity, and reproductive and developmental effects. This broadened scope will enhance the NTP's opportunities for gaining knowledge about exposure-related non-cancer disease etiologies and dysfunction(s) and enrich the science base available to regulatory agencies for assessment of human risk.

Development of Registries

Understanding the impact of environmental exposures on reproduction and development requires that efforts be directed toward assessing both exposures and effects in humans. More extensive birth registries are needed for determining the types, frequencies, and geographic distribution of birth defects, and efforts are needed for gathering reliable information about children’s exposures to environmental agents. Such information would greatly facilitate future research initiatives and provide important and useful human exposure information for the CERHR’s use in selecting chemicals for evaluation and in assessing the potential adverse effects on reproduction and development from those chemical exposures.

RESPIRATORY TOXICOLOGY

Current Research Initiatives

Inhalation exposure to environmental and occupational toxicants is a major contributing factor to human health problems. Chemicals being studied or planned for laboratory study by inhalation routes include 1-bromopropane, carbonyl sulfide, cobalt, cumene, decalin, diethylamine, divinylbenzene, α-methylstyrene, methyl isobutyl ketone, propargyl alcohol, propylene glycol mono-*t*-butyl ether, stoddard solvent (type IIc), and vanadium pentoxide (see Tables 9, 12 and 14. Several agents are also under consideration as causative for adult or childhood respiratory diseases through epidemiology studies (see page 64).

Inhalation Facilities and Methods Development

Many NTP research efforts are directed toward understanding the biochemical and molecular mechanisms of toxicity of inhaled chemicals. The activities within the inhalation facilities at NIEHS/NIH and NIOSH/CDC provide support for NTP-sponsored inhalation studies by conducting special studies on inhalation dosimetry and mechanisms of toxicity. Additional activities include development of inhalation exposure technology and models for investigating pulmonary disease (Frazer, NIOSH/CDC; Moorman, NIEHS/NIH). At the NIOSH/CDC facility, new inhalation exposure systems have been developed for small rodents for the following toxicants: toluene diisocyanate, metal working fluids, endotoxin, ozone, latex protein and powder, methacholine, and popcorn flavoring vapor. Currently welding fume and glass fiber exposure systems are under development (Frazer, NIOSH/CDC).

Exposure to isocyanates is known to cause occupational asthma. The development of a method to identify and measure isocyanate species in air by derivatization with the MAP reagent and analysis by liquid chromatography/mass spectrometry is underway. The goal of this project is to enable measurement of complex isocyanate mixtures in situations where bulk isocyanate products may not be available as qualitative standards (Streicher, NIOSH/CDC).

Exposure of laboratory animals by inhalation closely duplicates the way that humans are exposed to airborne toxicants and is essential for studying the role of chemicals in respiratory disease; however, inhalation studies are technologically difficult to perform and require unique equipment and resources. A prototype whole-body vibration system for small laboratory animals has been developed at the NIOSH/CDC and currently is being tested for use in studying whether whole-body vibration affects aerosol deposition and gas absorption in the lungs. This study will examine specific pulmonary functions of laboratory rodents and guinea pigs exposed to toxic aerosols and gases. Future plans include modifying the system to accommodate multiple animals and integrating existing inhalation exposure methods (Frazer, NIOSH/CDC).

The study of occupational asthma often involves inhalation studies using laboratory animals. Scientists at NIOSH/CDC are developing non-invasive methods for measuring the physiological response(s) of small laboratory animals exposed to airborne toxicants and workplace dusts known to cause asthma. Methods to be developed include: estimates of airway resistance, measurements of the acoustic properties of the thorax, 1/f noise analysis of breath signals, and analysis of cough sounds of small animals exposed to asthma-causing agents. Such methods will permit repeated monitoring over extended periods allowing more efficient use of animals and potentially reducing animal numbers (Reynolds, NIOSH/CDC).

Inhaled xenobiotics often affect the ventilation mechanics of test animals, and physiological modeling and dosimetry rely heavily on an estimate of the rate of alveolar ventilation. A whole body plethysmograph has been developed at the NIEHS/NIH to use with the nose only exposure systems for measuring the minute alveolar volume in test animals under exposure conditions. This information is being used in conjunction with disposition and toxicity data in a physiological simulation to build a physiologically realistic description of the toxicokinetics of compounds under study; a toxicokinetic model to describe the inhalation of styrene in rats and mice has been developed (Moorman, NIEHS/NIH).

Magnetic resonance imaging (MRI) provides an opportunity to visualize internal organs at microscopic resolution in live animals and to conduct specialized studies on fixed blocks of pathologic specimens. Techniques at the NIEHS/NIH have been refined for use of hyperpolarized helium in imaging fine structures of the lung in live animals. Using the elastase rat lung model for emphysema, data are currently being collected using hyperpolarized helium as well as standard proton images for assessment of pulmonary damage and pulmonary function. Future efforts include studies of experimentally-induced emphysema in the rat model to establish reproducibility of the model and the parameters and sensitivity of the MRI techniques and studies on environmentally induced or exacerbated emphysema in animal models. The effect of environmentally relevant particulates on lung pathophysiology will be studied in collaboration with EPA (Maronpot, NIEHS/NIH).

Lung Disease Susceptibility

Several efforts at NIOSH/CDC are working to understand how the inhalation of particulates can affect susceptibility of workers to lung disease. One study is evaluating the physical and chemical characteristics of respirable particles (quartz, diesel exhaust, and hard metal process materials) and testing their interactions with pulmonary surfactant and surfactant components. Particle properties will be related to toxicological properties. Subsequent studies will address how the physical and chemical properties of these particles might affect disease etiology (Wallace, NIOSH/CDC). Another project is trying to understand how exposure to inhaled metal-containing particles affects susceptibility to pulmonary infections. This study is focusing on understanding the mechanisms of how workers from such industries as welding and construction become more susceptible to infection than the general population. A variety of metal-containing particles (*e.g.*, residual oil fly ash or welding fumes) differing in their inflammatory and fibrotic characteristics are being evaluated for toxic and immune effects in the lungs of rodents infected *in vivo* with *Listeria monocytogenes*. Additional studies will assess *in vitro* and *ex vivo* the effects of these particles on alveolar macrophage function (Antonini, NIOSH/CDC).

Using *in vitro* and *in vivo* models, NIOSH/CDC is investigating the role of diisocyanates-thiol reaction products in mediating diseases commonly seen in polyurethane user industries as a result of inhalation exposure to isocyanates. Results suggest that diisocyanate-thiol interactions may play a role in subsequent toxic or hypersensitivity reactions. Future efforts will focus on studying these reactions and understanding their role in the disease process (Siegel, NIOSH/CDC).

Exposure to nuisance dusts, *i.e.*, particles not otherwise classified, is widespread in industrial, construction, agricultural, and mining settings. Investigators at NIOSH/CDC are testing the hypothesis that lung injury results form a defect in clearance of apoptotic cells that are induced when the lung burden to inhaled particles reaches a sufficient level. Initial studies are focusing on clarifying dose-response relationships between apoptotic cell number and lung injury. Subsequent studies will examine the relationship of apoptosis and lung injury following inhalation exposure to titanium dioxide and field dusts (Mercer, NIOSH/CDC).

Another study is investigating whether endocrine status may alter susceptibility to occupational hazards that might be inhaled and if so, how. This research is focusing on the impact of female reproductive status (pregnancy and lactation) or thyroid hormone status on pulmonary immune responses to hazardous agents in rodents. Preliminary findings suggest that susceptibility of the lung to damage from airborne microbial products may be increased during pregnancy and that thyroid hormone status modulates pulmonary responses to biogenic substances and oxidant gases. Studies investigating the underlying mechanisms of these effects are underway (Huffman, NIOSH/CDC).

Asphalt fume exposure has been associated with airway irritation and hyperactivity in some pavers. The effects of asphalt fumes on airway irritation, pulmonary inflammation, airway reactivity, and lung damage in a rodent model are currently being investigated. Using knowledge gained from a direct evaluation of asphalt paving conditions with the design and construction of a generator, this study is able to recreate exposures similar to those generated during road paving. Preliminary results from this inhalation study suggest that exposure does not cause significant inflammation or lung injury, but may affect metabolic function in the lung as measured by pulmonary P450 protein levels and activities. Companion studies of exposure to diesel exhaust particulates show affects on P450 activity. Future studies are planned to evaluate dose-response relationships for asphalt fume exposure (Ma, NIOSH/CDC).

Fibrosis

A key feature of environmental fibroproliferative diseases is fibroblast hyperplasia. Researchers at the NIEHS/NIH are investigating the mechanisms for fibrosis caused by the fibrogenic metal, vanadium pentoxide. Vanadium stimulates a wide spectrum of inflammatory mediators including cytokines, growth factor receptors, and intracellular signaling intermediates. Future work will focus on elucidation of molecular events and the development of intervention strategies (Bonner, NIEHS/NIH).

The NIOSH/CDC is developing spectroscopy-based imaging methods to detect and measure *in vivo* biochemical processes and tissue property changes that may provide earlier or improved diagnosis and evaluation of occupational injury or disease. A positron emission tomographic (PET) novel tracer for pulmonary silicosis is being tested in an animal model. Autoradiography studies will be conducted to determine the specificity of the label to fibrosis or inflammation (Wallace, NIOSH/CDC).

Areas for Future Initiatives and Resources

The NTP through its NIEHS/NIH – NIOSH/CDC interagency agreement (see page 23) is making a major effort toward creating in a laboratory setting certain occupational exposures and evaluating the impact of those occupational exposures on worker health. The initial efforts under this initiative are successful (*i.e.*, worker exposure to asphalt fumes and cellulose inhalation) and studies are moving forward on 1-bromopropane. The NTP plans to continue to evaluate other occupational exposures (*e.g.*, metal working fluids and welding fumes). The NTP also needs to address how it will use knowledge learned from these studies to communicate its findings and improve worker education.

CARCINOGENESIS

Current Research Initiatives

Chronic Phase of Study

Two-year studies in rodents remain the primary laboratory method by which chemicals or physical agents are identified as having the potential to be hazardous to man. These long-term toxicology and carcinogenesis studies (bioassays) in rodents generally employ both genders of rats (Fisher 344) and mice (B6C3F1 hybrid) with three exposure levels plus untreated controls in groups of 50 animals for two years; fish models are used occasionally. If adequate data exist in the literature for one rodent species, then typically only the remaining species is studied. The NTP interfaces its testing with regulatory agencies and the private sector in order to minimize duplication of effort. Table 14 lists the chemicals currently in the chronic phase of NTP study. Testing for 46 compounds is ongoing from FY 2001; 10 studies are scheduled to end with preparation of Technical Reports for review in October 2001 (2,4-Hexadienal, Riddelliine, and Vanadium Pentoxide) and September 2002 (6 reports, see page 16). Studies for 8 chemicals are scheduled to start in FY 2002 and awards are anticipated for future study of 8-10 additional chemicals per year. Several studies including urethane, ethanol, leucomalachite green and malachite green are being conducted at NCTR/FDA.

Table 14. Compounds in the Chronic Phase of NTP Study

Chemical Name	CAS No.	Project Leader[1]	Contract Project Officer[2]	Species: Strain	Route	Special Studies[3]	Study Length[4]
Studies ongoing in FY2002 as of 10/09/01							
AZT transplacental carcinogenesis study	30516-87-1	Rao	Rao	Mice: Swiss CD-1	In utero		19 months
Benzophenone	119-61-9	Chhabra	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Feed	Toxicokinetics: 2 weeks; 3, 12, and 18 months	2 years
2,2-bis(Bromomethyl)-1,3-propanediol (Fish project)	3296-90-0	Boorman	Bernheim	Fish: Medaka (Oryzias Latipes) Fish: Guppy (Poecilia Reticulata)	Aqueous Exposure		9 months; 16 months
Bromodichloromethane (Water DBP)	75-27-4	Melnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Water		2 years
Bromochloroacetic Acid (Water DBP)	5589-96-8	Melnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Water		2 years
trans-Cinnamaldehyde	4371-10-9	Hooth	Orzech	Mice: B6C3F1 Rats: Fischer 344	Microencapsulated in feed	Toxicokinetics: 2 weeks; 3, 12, and 18 months	2 years
Cumene	98-82-8	Chan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation		2 years
Decalin	91-17-8	Chan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation		2 years
Dibromoacetonitrile (Water DPB)	3252-43-5	Melnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Water		2 years
Dibromoacetic acid (Water DBP)	631-64-1	Melnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Water		2 years
Diisopropylcarbodiimide	693-13-0	Chhabra	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Topical		2 years
Dipropylene glycol	25265-71-8	Chhabra	Orzech	Mice: B6C3F1 Rats: Fischer 344	Water		2 years
Divinylbenzene	1321-74-0	Morgan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation		2 years
Elmiron (Sodium pentosanpolysulfate)	37319-17-8	Abdo	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage		2 years
Ethanol	64-17-5	Beland (NCTR)	Bucher	Mice: B6C3F1	Water	DNA adducts, cell proliferation, apoptosis	2 years
Formamide	75-12-7	Irwin	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage		2 years
2,4-Hexadienal	142-83-6	Chan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Gavage	DNA adduct analysis of forestomach and liver in males	2 years
α-Hydroxy acid (Salicylic acid)	50-21-5	Howard (NCTR)	Bucher	Mice: SKH-1	Topical		1 year

Chemical Name	CAS No.	Project Leader[1]	Contract Project Officer[2]	Species: Strain	Route	Special Studies[3]	Study Length[4]
β-Hydroxy acid (glycolic)	79-14-1	Howard (NCTR)	Bucher	Mice: SKH-1	Topical		1 year
5-Hydroxymethyl-furfural	67-47-0	Irwin	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage	Special histology of GI tract	2 years
Leucomalachite green	129-73-7	Culp (NCTR)	Bucher	Mice: B6C3F1 Rats: Fischer 344	Feed		2 years
Malachite green	569-64-2	Culp (NCTR)	Bucher	Mice: B6C3F1 Rats: Fischer 344	Feed		2 years
Methylene blue trihydrate	7220-79-3	Chhabra	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Gavage	Toxicokinetics: 4 and 13 weeks and 19 months	2 years
2-Methylimidazole	693-98-1	Chan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Feed	Thyroid hormones; Liver P450s and UDP-glucose; 20/sex/ species/group at 8 days and 13 weeks	2 years
4-Methylimidazole	822-36-6	Chan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Feed		2 years
Methyl isobutyl ketone	108-10-1	Chhabra	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation		2 years
α-Methylstyrene	98-83-9	Morgan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation		2 years
Nitromethane (Fish project)	75-52-5	Boorman	Bernheim	Fish: Medaka (Oryzias Latipes) Fish: Guppy (*Poecilia Reticulata)*	Aqueous exposure		9 months; 16 months
Propargyl alcohol	107-19-7	Hooth	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation		2 years
Propylene glycol mono-*t*-butyl ether	57018-52-7	Herbert	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation	Toxicokinetics: 2 and 6 weeks; 3, 6, and 12 months for urine; 2, 6, and 13 weeks for blood	2 years
Riddelliine	23246-96-0	Chan & Chou (NCTR)	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Gavage	DNA adduct analysis of female rat livers; oncogene	2 years
Simazine	122-34-9	Trnovec	Boorman	Mice: B6C3F1	Feed		2 years
Sodium chlorate	7775-09-9	Melnick	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Water	At days 4 & 21, and at 13 weeks thyroid hormones/ pathology; 10/sex/species	2 years
Stoddard solvent (Type IIC)	64742-88-7	Chhabra	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation	Rats: 10/sex group kidney cell proliferation and α2μ-globulin	2 years
Triethanolamine	102-71-6	Bucher	Orzech	Mice: B6C3F1	Topical		2 years
1,2,3-Trichloropropane (Fish	96-18-4	Boorman	Bernheim	Fish: Medaka (*Oryzias Latipes*)	Aqueous		9 months; 16

Chemical Name	CAS No.	Project Leader[1]	Contract Project Officer[2]	Species: Strain	Route	Special Studies[3]	Study Length[4]
project)				Fish: Guppy (*Poecilia Reticulata)*	exposure		months
Urethane (ethyl carbamate)	51-79-6	Beland (NCTR)	Bucher	Mice: B6C3F1	Water	DNA adducts; cell proliferation/apoptosis at same dose levels	2 years
Urethane + Ethanol (combination)	URETHCOMB	Beland (NCTR)	Bucher	Mice: B6C3F1	Water	DNA adducts; cell proliferation/apoptosis at same dose levels	2 years
Vanadium pentoxide	1314-62-1	Roycroft	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation	Toxicokinetics: 3, 6, 12, and 18 months; special female rat and mouse study (40 mice or rats/treated group)	2 years
Binary mixture (TEFEvaluation)	TEFBINARYMIX	Walker	Orzech	Rats: Sprague Dawley	Gavage	T3, T4, TSH, P450s; Toxicokinetics	2 years
Dioxin mixture (TEFEvaluation)	TEFDIOXINMIX	Walker	Orzech	Rats: Sprague Dawley	Gavage	T3, T4, TSH, P450s; Toxicokinetics	2 years
PCB 153: 2,2'-4,4',5,5'-Hexachlorobiphenyl (TEFEvaluation)	35065-27-1	Walker	Orzech	Rats: Sprague Dawley	Gavage	T3, T4, TSH, P450s; Toxicokinetics; stop study	2 years
PCB mixture PCB118/PCB126 (TEFEvaluation)	TEFPCBMIX	Walker	Orzech	Rats: Sprague Dawley	Gavage	T3, T4, TSH, P450s; Toxicokinetics; stop study	2 years
PCB-126: 3,3',4,4',5-Pentachlorobiphenyl (TEFEvaluation)	57465-28-8	Walker	Orzech	Rats: Sprague Dawley	Gavage	T3, T4, TSH, P450s; Toxicokinetics; stop study	2 years
PCDF: Pentachlorodibenzofuran (TEFEvaluation)	57117-31-4	Walker	Orzech	Rats: Sprague Dawley	Gavage	T3, T4, TSH, P450s; Toxicokinetics; stop study	2 years
TCDD: 2,3,7,8-Tetrachloro-dibenzo-*p*-dioxin (TEFEvaluation)	1746-01-6	Walker	Orzech	Rats: Sprague Dawley	Gavage	T3, T4, TSH, P450s; Toxicokinetics; stop study	2 years
Studies proposed to start in FY 2002 as of 10/09/01							
bis(2-Chloroethoxy)methane	111-91-1	Dunnick	Orzech	Mice: B6C3F1 Rats: Fischer 344	Topical		
Chromium picolinate monohydrae (CrIII)	27882-76-4	Abdo	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Feed		2 years
Cresol (meta/para mixture)	1319-77-3	Chhabra	Orzech	Mice: B6C3F1 Rats: Fischer 344	Feed		
1,2-Dibromo-2,4-dicyanobutane	35691-65-7	Dunnick	Orzech	Mice: B6C3F1 Rats: Fischer 344	Topical		
Isoeugenol	97-54-1	Abdo	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage		
β-Myrcene	123-35-3	Chan	Orzech	Mice: B6C3F1	Gavage		2 years

Chemical Name	CAS No.	Project Leader[1]	Contract Project Officer[2]	Species: Strain	Route	Special Studies[3]	Study Length[4]
				Rats: Fischer 344			
Sodium Dichromate dihydrate (CrVI)	7789-12-0	Abdo	Ress/Roycroft				2 years
3,3',4,4'-Tetrachlorazobenzene	14047-09-7	Hooth	Orzech	Mice: B6C3F1 Rats: Sprague-Dawley	Gavage		
Studies to end in FY 2002 as of 10/09/01							
trans-Cinnamaldehyde	14371-10-9	Bucher	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Micro-encapsulated in Feed	Toxicokinetics: rats at 2 weeks, 3, 12, and 18 months	2 years
Decalin	91-17-8	Chan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Inhalation		2 years
Dipropylene glycol	25265-71-8	Chhabra	Orzech	Mice: B6C3F1 Rats: Fischer 344	Water		2 years
Elmiron (Sodium pentosanpolysulfate)	37319-17-8	Abdo	Orzech	Mice: B6C3F1 Rats: Fischer 344	Gavage		2 years
Ethanol	64-17-5	Beland (NCTR)	Bucher	Mice: B6C3F1	Water	DNA adducts, cell proliferation, apoptosis	2 years
2,4-Hexadienal	142-83-6	Chan	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Gavage	DNA adduct analysis of forestomach and liver in males	2 years
Riddelliine	23246-96-0	Chan & Chou (NCTR)	Roycroft	Mice: B6C3F1 Rats: Fischer 344	Gavage	DNA adduct analysis of female rat livers; oncogene	2 years
Urethane (Ethyl carbamate)	51-79-6	Beland (NCTR)	Bucher	Mice: B6C3F1	Water	DNA adducts; cell proliferation/apoptosis at same dose levels	2 years
Urethane + Ethanol (combination)	URETHCOMB	Beland (NCTR)	Bucher	Mice: B6C3F1	Water	DNA adducts; cell proliferation/apoptosis at same dose levels	2 years
Vanadium pentoxide	1314-62-1	Roycroft	Roycroft	Mice: B6C3F1Rats: Fischer 344	Inhalation	Toxicokinetics: 3, 6, 12, and 18 months; special female rat and mouse study (40 mice or rats/treated group)	2 years

[1] Project Leader - NIEHS/NIH staff scientist (unless otherwise indicated) who oversees each chemical's evaluation
[2] Contract Project Officer - NIEHS/NIH staff scientist (unless otherwise indicated) who coordinates research activities with the contract laboratory
[3] Special Studies:
T3 = Thyroxine 3 and T4 = Thyroxine 4
P450 = Cytochrome P450 enzymes
TSH = Thyroid stimulating hormone
TK = Tyrosine kinase
[4] Standard evaluations include necropsy evaluation and histopathologic examination of tissues.

Radiofrequency Emissions Associated from Cellular Telephones

In response to a nomination from the FDA, the NTP plans to conduct laboratory research to help clarify any potential health hazard for the U.S. population from radiofrequency radiation emissions used in cellular telephone transmissions (see page 17).

Hexavalent Chromium

In response to public concern in California, the NTP is conducting short- and long-term studies of hexavalent chromium administered to laboratory animals as sodium dichromate dihydrate in drinking water, as well as studies on hexavalent chromium's tissue absorption. Data from these studies is available on the NTP web site (http://ntp-server.niehs.nih.gov, see What's New). Additional details are available on page 21 (Abdo, NIEHS/NIH).

Mechanism-Based Carcinogenesis

Cell Cycle Control

Exposure to environmental carcinogens can result in loss of normal cellular growth control and eventual tumor formation. A variety of activated oncogene (*e.g., ras, mos,* MEK) products and tumor suppressor gene products (*e.g.,* ataxia telangiectasia, RB and p53) interact directly or indirectly with vital cell cycle control signal transduction pathways.

The p53 protein is involved in regulation of the cell cycle, apoptosis and cell differentiation. Inactivation of the p53 tumor suppressor gene is the most common genetic abnormality of human cancers. As a transcription factor, p53 is important for maintaining genomic integrity and regulating cell growth and death. Alterations of the p53 gene may result from spontaneous mutation or various environmental factors such as radiation or chemical exposures. Post-translational modification is the major mechanism for regulating p53 induction and activation, primarily by phosphorylation and acetylation. Efforts are ongoing at the NIEHS/NIH to study how changes in p53 phosphorylation relate to its function and to develop methods for assessing p53 phosphorylation status (Merrick, NIEHS/NIH). Studies at NCTR/FDA in the p53 transgenic mouse are investigating the role of the p53 tumor suppressor gene in the evaluation of genistein, an estrogenic compound of soy products (Morris, NCTR/FDA).

Preliminary studies at NIOSH/CDC have demonstrated that two novel proto-oncogenes (translation initiation factor 3 and translation elongation factor 1δ) are over-expressed in cadmium-induced carcinogenesis. Studies are being conducted to understand the underlying molecular mechanism(s) responsible for the oncogenic potential of these factors, determine whether this over-expression also occurs in transformed or tumor cells induced by other occupational carcinogens (*e.g.,* beryllium and tetrachloroethylene), and determine whether mutations or epigenetic changes in these genes are responsible for this over-expression. Interactions between these novel genes and genes involved in cell cycle control will also be investigated to determine the mechanisms responsible for the cell transformation and tumorigenesis caused by over-expression of these novel genes (Ong, NIOSH/CDC).

Cytotoxicity

Furan is the parent structure of a large class of naturally occurring and synthetic compounds. It is hepatocarcinogenic and hepatotoxic, but not mutagenic or directly DNA reactive; therefore, furan is considered a model agent for studying the dose-response characteristics and mechanisms of action of cytotoxic carcinogens. The NIEHS/NIH is undertaking a study to determine if non-cytotoxic doses of furan induce liver neoplasms in female B6C3F1 mice. Data from this study will be used to evaluate relationships between dose, cytotoxicity, compensatory cell growth, and tumor induction by a model hepatic cytotoxic carcinogen (Maronpot, NIEHS/NIH).

Oxidative Stress

Researchers at NIOSH/CDC are studying carcinogenic mechanisms of occupational exposures. Several transition metals such as chromium(VI), nickel(II), vanadium(V) and cobalt(II) are established carcinogens and each is able to generate reactive oxygen species upon reaction with cells. The role of free radical reactions leading to carcinogenesis is currently being investigated *in vitro* and will be expanded to *in vivo* studies in the future (Shi, NIOSH/CDC).

Peroxisome proliferators have been shown to produce hepatocarcinomas in rodents. Preliminary studies using the peroxisome proliferator, Wyeth-14643 (Wy), implicate the production of fatty acid metabolites by cyclooxygenase isoform, COX-1, in non-parenchymal cells with subsequent hepatocyte metabolism of these products in the formation of mitogenic metabolites. Additional experiments are in progress to confirm these findings and further characterize the roles of hepatocytes and non-parenchymal cells in Wy-induced liver tumors (Ghanayem, NIEHS/NIH).

Genetic Toxicology and Mutagenesis

Genetic toxicity test results are used in making decisions about whether a substance should be tested for carcinogenicity in rodents; to aid in the interpretation of toxicity, carcinogenicity, or other *in vivo* test results; and to provide a database for use in structure-activity analyses. Testing is conducted at contract laboratories. Testing in the first quarter of FY2002 is being started for 20 chemicals using the *in vitro Salmonella typhimurium* assay (Table 15); testing capacity is set at 50 chemicals. During this quarter testing of 45 substances will be completed (data not shown).

Gene mutations and DNA damage are examined in most tissues; cytogenetic effects, measured as the induction of micronuclei, are generally examined in bone marrow cells or in peripheral erythrocytes. The erythrocyte studies are integrated with other toxicity evaluations to minimize the use of animals and to expand the toxicology information for the chemical in the same animals. The testing of micronuclei in peripheral blood erythrocytes will be started for 11 substances in the first quarter of FY 2002 (Table 15) (Caspary, NIEHS/NIH). During this quarter testing of 9 substances will be completed (data not shown).

Genetic toxicity information obtained on chemicals tested by the NTP is forwarded to the Human Genome and Toxicology Group at Oak Ridge National Laboratory, Oak Ridge, Tennessee for inclusion in the Environmental Mutagen Information Center's computerized database. Information within this database is a part of National Library of Medicine's TOXNET and is available to the scientific community through the library's TOXLINE.

Table 15. Compounds Being Evaluated for Genetic Toxicity[1]

Chemical	CAS No.	Testing Battery[2]
Studies started in FY 2002 – October 1, 2001 – March 31, 2002		
Aniline	62-53-3	Micronucleus
3'-Azido-3'-deoxythymidine + 2',3'-Dideoxyinosine	AZTDDICOMB	Micronucleus
Benzene	71-43-2	Micronucleus
Benzene + aniline combination	BENZANILINMX	Micronucleus
Clarithromycin	81103-11-9	Micronucleus
Diazoaminobenzene	136-35-6	Micronucleus
Diphenolic acid	126-00-1	Micronucleus
Isoleugenol	97-54-1	Micronucleus
Methylene blue trihydratae (C.I. Basic blue 9)	7220-79-3	Micronucleus
Octabromodiphenyl ether	32536-52-0	Micronucleus
Pentabromodiphenyl oxide	32534-81-9	Micronucleus
2-Ethylhexyl-*p*-dimethylaminobenzoic acid	21245-02-3	Salmonella
S-adenosylmethionine	29908-03-0	Salmonella
S-adenosylmethionine chloride	24346-00-7	Salmonella
α/β-Thujone mix	THUJONEMIXAB	Salmonella
Apigenin	520-36-5	Salmonella
Bladderwrack (Herbal)	68917-51-1	Salmonella
Bladderwrack extract (Herbal)	84696-13-9	Salmonella
Chlorodifluoromethane	75-45-6	Salmonella
Cylindrospermopsin	143-545-90-8	Salmonella
Dibenzofuran	132-64-9	Salmonella
Diphenolic acid	126-00-1	Salmonella
Epigallocatechin-3-gallate	989-51-5	Salmonella
Grape seed extract (herbal medicine)	GRAPESEEDEXT	Salmonella
2,2',4,4',5,5'-Hexabromodiphenyl ether	68631-49-2	Salmonella
Metal working fluids	METALWORKMIX	Salmonella
Methyl soyate	67784-80-9	Salmonella
2-Methyl tetrahydrofuran	96-47-9	Salmonella
Octabromodiphenyl ether	32536-52-0	Salmonella
Pentabromodiphenyl oxide	32534-81-9	Salmonella
2,2',4,4'-Tetrabromodiphenyl ether	5436-43-1	Salmonella

[1]Studies being conducted in the first quarter of FY 2002

[2] Testing Battery

Micronuclei: Induction of micronuclei (MN) in peripheral blood erythrocytes of mice. Mice are treated with chemical for 90 days or 26 weeks depending upon the strain being tested. When a chemical is tested in short term (>14 day) toxicity studies in mice, blood smears are made at the time of animal sacrifice. These smears are coded and sent to a contract laboratory to be scored for the presence of micronuclei in the peripheral blood erythrocytes. Blood smears from males and females are scored.

Salmonella Mutagenicity Test: Up to eight Salmonella tester strains (TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, and TA1538) are used in a pre-incubation modification of the Ames Salmonella/microsome test. Volatile chemicals and gasses are tested in a desiccator. Exogenous metabolic activation is provided by liver homogenates from Aroclor 1254-induced male Sprague-Dawley rats and Syrian hamsters. Testing is performed so that if a chemical is mutagenic in strains TA98 or TA100, it will not be tested in other strains, unless specific questions concerning the test chemical's spectrum of activity are to be addressed. If the chemical is not mutagenic in strains TA98 or TA100, it is tested in strains TA97 and TA1535. If the chemical is negative or equivocal in any of these strains or depending on the chemical's structure and presumed active products, it may also be tested in one or more of the other tester strains.

Molecular Evaluations of Neoplasms

Knowledge about the spectrum of genetic alterations in chemically induced rodent tumors, their temporal appearance in progression from pre-neoplastic lesions to neoplasms, and the way in which these factors differ among tissues and among chemicals may provide a molecular basis for distinguishing between spontaneously and chemically-induced neoplasms. A systematic effort is being made at the NIEHS/NIH to identify alterations in oncogenes and tumor suppressor genes in the most frequent sites of spontaneous and chemically induced neoplasms from F334 rats and B6C3F1 mice used in chronic bioassays. DNA isolated from neoplasms in control and chemically treated rodents from prospective and reprospective studies are analyzed for genetic alternations using PCR-based assays. These studies are designed to correlate chemical-specific properties (*e.g.*, structure, genotoxicity, metabolism) with characteristics of the spectrum of genetic alterations present in preneoplastic and neoplastic lesions of target organs (Sills, NIEHS/NIH).

Point mutations in *K-ras* were found in lung neoplasms from B6C3F1 mice treated with 2,2-bis(bromomethyl)-1,3-propanediol (BMP). Preliminary studies examined lung tumors for loss of heterozygosity on distal chromosome 6 (the site of a major lung susceptibility locus in the region of the *K-ras* gene) and allelic imbalances were detected in BMP-induced lung neoplasms. These studies are being extended to include evaluation of ozone-induced neoplasms and BMP-induced Harderian gland neoplasms. Forestomach tumors induced by a class of carcinogens (chloroprene, isoprene, and 1,3-butadiene) are being examined for activating mutations in the *ras* gene. This information will be related to the known mutagenic properties of exogenously and endogenously induced DNA adducts to provide information about the relevance of different adducts. Mutational spectra from other sites will be compared to that of the forestomach to determine whether a similar mechanism is involved (Sills, NIEHS/NIH). Another study has found that tetrafluoroethylene-induced hepatocellular neoplasms most likely develop by a pathway independent of *H*- and *K-ras* proto-oncogene activation (Sills, NIEHS/NIH). Cobalt sulfate induces a higher incidence of alveolar/bronchiolar adenomas and carcinomas in B6C3F1 mice as compared to controls. Unique point mutations have been detected in *K-ras* exon 2. Future studies will focus on determining the mechanism for these mutations and characterizing genetic alternations in potential tumor suppressor genes that might be relevant to human exposure. Currently, comparisons are being made between cobalt sulfate-induced lung neoplasms and other neoplasms (Sill, NIEHS/NIH).

It is hypothesized that epoxide intermediates play a role in the pathogenesis of hemangiosarcomas by causing genetic alterations in tumor suppressor genes and proto-oncogenes. In the B6C3F1 mouse, chemicals such as chloroprene, 1,3-butadiene, *o*-nitrotoluene, and tetrafluoroethylene cause hemangiosarcomas in the liver and other organ systems. These tumors are being evaluated for genetic alterations in the *p53* gene (Sills, NIEHS/NIH).

Researchers at NCTR/FDA are examining genetic polymorphisms as markers for potential genotoxicity. Current efforts are directed toward characterizing DNA adducts from tamoxifen metabolites and analogues and determining if tamoxifen or its derivatives increases the frequency of mutations at the *Hprt* gene and if these mutations can be biomarkers for potential genotoxicity of anti-estrogens. Other studies have found that abnormal folate metabolism is associated with polymorphisms in the methylene tetrahydrofolate reductase and methionine synthase reductase genes in mothers of children with Down's syndrome. Follow-up studies are underway to determine if DNA hypomethylation secondary to inadequate maternal folate might contribute to abnormal chromosomal segregation and non-disjunction of chromosome 21 (Beland/Kadlubar/James, NCTR/FDA).

NCTR/FDA scientists conduct fundamental research aimed at defining the pathways from initial DNA damage to mutation and such research centers on the development and validation of new *in vitro* and *in vivo* methodologies by which to assess genetic risk. The understanding of mutational mechanisms, combined with test systems with an increased capability to detect genetic damage, provides regulatory agencies with the most current knowledge on which to base regulatory decisions (Moore, NCTR/FDA).

Noncordance of Mutagenicity and Carcinogenicity Assays

The NIEHS/NIH is interested in those chemicals that produce positive mutagenicity *in vitro* and fail to produce carcinogenicity in NTP bioassays. Chemicals that fail to cause cell proliferation in the early stages of chemical exposure also fail to cause carcinogenesis regardless of their activity in mutagenesis assays. The NIEHS/NIH has determined that the Big Blue transgenic rodent mutation assay can detect mutations derived from a carcinogen that is nonmutagenic *in vitro* and can distinguish between the mutation spectrum from treated mice compared to control mice. Future studies will evaluate the effects of 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin, dietary overload to iron, and tamoxifen (a nongenotoxic carcinogen and reproductive toxicant) (Cunningham, NIEHS/NIH).

Big Blue lacl assays measure gene mutations in somatic cells and are sensitive to fundamentally different spectra of mutagenic events than are detected in the micronucleus assay. In the Big Blue rat model, the lacl gene is integrated into the genome of every cell of the animal and can be retrieved as a reporter gene for mutational analysis. The lacl assay has the major advantage of being able to measure the mutations (base pair substitutions, frameshifts and small deletions) in any tissue from which DNA can be isolated. Moreover, mutant induction in a neutral reporter gene, like lacl, can accumulate with time during chronic exposure and, thus, be a sensitive indicator of genotoxic damage (Manjanatha, NCTR/FDA). At NCTR/FDA, studies are underway investigating the mutagenicity of malachite green, and its primary metabolite, leucomalachite green, in the Big Blue rat and mouse models, where the *lacl* gene is integrated into the genome of every cell in the animal. The gene can be retrieved as a reporter gene for mutational analyses (Heflich, NCTR/FDA).

Peroxisome proliferators

As a class, peroxisome proliferators are often rodent hepatic, testicular, or pancreatic carcinogens and are thought to act through a cell proliferative, promotional, and/or oxidative stress mechanism(s). The NIEHS/NIH is interested in the relevance of peroxisome proliferating chemical induction of rodent neoplasia for humans. Interspecies comparisons are being conducted using sensitive (rats and mice) and insensitive (hamsters) species. The peroxisome proliferators being evaluated include Wyeth-14643, dibutyl phthalate, 2,4-dichlorophenoxyacetic acid, and gemfibrozil. Through R03 grants, the NIEHS/NIH is involving extramural scientists to examine molecular and biochemical mechanisms relevant to the carcinogesis of peroxisome proliferators (Cunningham, NIEHS/NIH).

Breast Cancer

Scientists at the NCTR/FDA are developing methods to assay hydroxylation of endogenous estrogens for possible use in screening for breast cancer risk. This effort is an outgrowth of clinical and experimental data that have shown differences in 2- and 4-hydroxylation of endogenous estrogen relative to risk for developing breast cancer (Beland, NCTR/FDA).

Lung Cancer

Lung cancer is the most common malignancy in the United States and is ranked second only to bladder cancer in the proportion of cases thought to be due to environmental exposures. Evidence indicates that there is a strong genetic component in susceptibility to adenocarcinoma of the lung, which is rapidly becoming the most common lung cancer type. Researchers at the NIEHS/NIH are continuing their efforts to identify murine lung tumor susceptibility genes and are concentrating on the *Par2* locus on chromosome 18. The goal is to determine if human homologues of these genes play a role in lung cancer. They have narrowed the locus to a small region and are working on identification of candidate genes in that region (Devereaux, NIEHS/NIH).

A comparative pathology project at NIOSH/CDC is examining the relationship between coal dust deposition in the lungs of miners and alterations in the biochemical pathways involved in the bio-activation of polycyclic aromatic hydrocarbons, such as those in cigarette smoke. The lung tissue from non-smoking and smoking miners is being evaluated for the interactions of smoking and coal dust exposure in the occurrence of alveolar epithelial cell hypertrophy and hyperplasia, pulmonary fibrosis, lung cancer, and cytochrome P4501A1 activity. A second component of this project includes biochemical and immunohistochemical studies of rodents that are exposed to the major components of respirable coal mine dust, including, crystalline silica, or iron oxide, in the presence and absence of β-naphthoflavone, an polycyclic aromatic hydrocarbon inducer of cytochrome P4501A1. Tissue from New Zealand White rabbits chronically exposed to intratracheal saline or silica followed by β-naphthoflavone is also being examined. Preliminary data suggest that various respirable dusts of coal mines are each modifiers of cytochrome P4501A1 induction by polycyclic aromatic hydrocarbons and current work is focusing on identifying their mechanism(s) of action. These results may help explain the variability in carcinogenicity observed in epidemiology studies of coal mine dust exposure (Hubbs, NIOSH/CDC).

Skin Cancer

Investigators at both NIOSH/CDC and NCTR/FDA are interested in learning how environmental factors and exposures affect susceptibility and development of skin cancer (Howard, NCTR/FDA; Ding, NIOSH/CDC; see page 19).

Transgenic Animal Evaluations

In addition to standard rodent models, transgenic animals are being used increasingly in NTP studies as part of the NTP's expanding efforts to understand the mechanism(s) of environmental toxicant induction of cancer and associated molecular genetics. Such information is critical for developing risk assessment procedures and strategies for intervention and prevention of environmental disease. Six chemicals are currently being studied for their carcinogenic potential using transgenic mouse models (Table 16). The data from previous studies using transgenic models is being evaluated for their utility in carcinogen hazard identification. As an initial effort, two technical reports from studies using transgenic models, tentaerythritol triacrylate and trimethylolpropane triacrylate, are scheduled for peer review in September 2002, (see page 16) and the NTP plans to begin 7 studies in FY 2002 of which 5 will be rapid screening studies for carcinogenesis.

The Tg.AC transgenic mouse skin model appears useful for studying the molecular mechanisms that underlie multi-stage tumorigenesis. Studies are underway to identify the common factors induced by chemicals, wound repair, or radiation that would lead to activation of the *v-Ha-ras* transgene. Efforts are focusing on identifying and characterizing epidermal stem cell markers to visualize and monitor the process of chemical- or wound-induced skin cancer development from the earliest stages of disease (Tennant, NIEHS/NIH).

Human and rodent trans-species carcinogens often demonstrate similar organotropic patterns of neoplasia and loss of heterozygosity. The NIEHS/NIH is investigating the mechanisms of carcinogen-induced DNA damage in the *p53* haploinsufficient mouse, specifically to identify the role of homolgous recombination, to determine loci-specific interference with recombination on chromosome 11 when animals are exposed to environmental carcinogens, and to evaluate the meiotic and mitotic recombination genotype patterns (French, NIEHS/NIH).

Table 16. Compounds Being Tested for Carcinogenic Potential using Transgenic Models

Chemical Name	CAS No.	Project Leader[1]	Contract Project Officer[2]	Species: Strain[3]	Route	Special Studies[4]	Study Length[5]
Studies ongoing in FY 2002 as of 10/09/01							
n-Acetylcysteine (Antioxidant study)	616-91-1	Maronpot	Maronpot	Mice: C57BL/6 PB-Transgene (TRAMP) Mice: C57BL/6	Gavage		12 weeks
Arsenic antioxidant mixture	7784-46-5	Germolec	Maronpot	Mice: Tg.AC (FVB/N) Hmz Mice: Tg.AC (FVB/N) Hom	Water		26 weeks
Epigallocatechin (Antioxidant study)	NAOSPINEXTR	Maronpot	Maronpot	Mice: C57BL/6 PB-Transgene (TRAMP) Mice: C57BL/6	Gavage		12 weeks
NAO (spinach extract) (Antioxidant study)	989-51-5	Maronpot	Maronpot	Mice: C57BL/6 PB-Tag Transgene (TRAMP) Mice: C57BL/6	Gavage		12 weeks
Ethinyl estradiol (Evaluation I)	57-63-6	Eastin	Chhabra/ Vallant	Mice: FVB/N Mice: Tg.AC (FVB/N) Hom	Gavage	Micronuclei	14 days; 26 weeks
Feed controls (Prevention 7)	PREVENTION7	Rao	Ney	Mice: MMTV/NEU (Tg.NK)	Feed		26-30 weeks
Studies proposed to start in FY 2002 as of 10/09/01							
Low isoflavone soy protein powder (Prevention 6)	ISOFLASOYPT	Rao	Ney	Mice: MMTV/NEU (Tg.NK)	Feed		26 weeks
Isoflavone concentrate (Prevention 6)	ISOFLAVCNCN	Rao	Ney	Mice: MMTV/NEU (Tg.NK)	Feed		26 weeks
Rapid screening studies for carcinogenicity							
PCB-126: 3,3',4,4',5-Pentachlorobiphenyl (TEFEvaluation)	57465-28-8			Mice: Tg.AC (FVB/N)	Gavage		
PCDF: Pentachlorodibenzofuran (TEFEvaluation)	57117-31-4			Mice: Tg.AC (FVB/N)	Gavage		
PCB-126 +PCDF				Mice: Tg.AC (FVB/N)	Gavage		
TCDD: 2,3,7,8-Tetrachloro-dibenzo-*p*-dioxin (TEFEvaluation)	1746-01-6			Mice: Tg.AC (FVB/N)	Gavage		
Senna	8013-11-4	Dunnick		Mice: p53 +/- (C57BL/6)			28 days; 26 and 39 weeks

[1] Project Leader: NIEHS/NIH staff scientist (unless otherwise indicated) who oversees each chemical's evaluation
[2] Contract Project Officer: NIEHS/NIH staff scientist (unless otherwise indicated) who coordinates research activities with the contract laboratory
[3] Hmz: hemizygous; Hom: homozygous
[4] Special Studies
Micronuclei: genetic toxicity testing. Induction of micronuclei in mouse peripheral blood erythrocytes. Mice are treated with chemical and blood smears are made at the time of animal sacrifice.
[5] Study Length
14 days or 17 days: Repeated dose study g used for determining the dose range for the subchronic study. The doses (usually five doses plus control) cover a wide dose range; 5 animals/group, 2 genders, 2 species; complete necropies; histopathologic evaluations on organs/tissues showing grow evidence of treatment-related lesions with corresponding tissues in control animals.
26 or 39 weeks: Transgenic carcinogenesis study to identify chemicals for carcinogenic potential. One or more transgenic stains may be used and the route of administration will depend upon the strain(s); generally 15 animals/group, 2 genders; complete necropies with histopathologic evaluation on all animals.

Antioxidants and Cancer

Prevention and intervention trials suggest that consumption of antioxidant-rich foods early in disease is protective against cancer while later intervention with dietary carotenoids/retinoids may exacerbate cancer. The NIEHS/NIH is conducting mechanism-based studies to investigate these paradoxical results. The Tg.AC mouse is an appropriate model to evaluate the effects of diet or the efficacy of anti-tumor agents or co-carcinogens that can modulate the tumor response. Inflammation (carcinogen or tumor-induced oxidative stress) is an intrinsic component to the progression of cancer. Studies are using Tg.AC models to examine modulation of oxidative stress by dietary antioxidants during various stages of pathogenesis (French, NIEHS/NIH).

Breast Cancer

Breast cancer is a multi-faceted disease that is influenced by many factors including genetics and environment. Some components of diet, such as vitamin A and its analogues (retinoids), and therapeutic agents, such as tamoxifen, may delay or prevent mammary cancer. The transgenic mouse model Tg.NK is being used at the NIEHS/NIH for evaluating the effects of dietary intervention strategies (*e.g.*, fiber, retinoids, melatonin, and linolenic acid) to delay mammary tumor development; results for all but linolenic acid were positive. Future plans include studies with dietary supplements, therapeutic agents, and less toxic combinations of previously tested agents. The NIEHS/NIH is also participating in a collaborative European Union project with the Danish Institute of Food Safety and Toxicology and the Laboratory of Health Effects Research, RIVM, The Netherlands using this animal model to study the effects of phytoestrogens in the prevention of breast cancer (Rao, NIEHS/NIH).

Epidemiology studies have established an association between occupation and environmental exposure to arsenic and skin, urinary bladder and lung cancers, and vascular diseases including atherosclerosis. A study at NIOSH/CDC is investigating the possible molecular and cellular mechanisms of arsenic-induced disease and the biomarkers that could be used in evaluating public health risks associated with exposure to arsenic. Preliminary data demonstrate that different pathways are involved in arsenic-induced gene expression in epithelial and endothelial cells. The role of nitric oxide and reactive oxygen species in arsenic-induced effects on endothelial cells and atherogenicity is under investigation (Luster, NIOSH/CDC).

Colon Cancer

Epidemiology studies indicate that regular use of non-steriodal inflammatory drugs (NSAIDs) reduces incidence of colorectal cancer by about 50% in humans. Likewise rodent models of colon cancer also show reductions in tumor formation following NSAID treatment. NSAIDs are thought to act by inhibition of cyclooxygenase-1 and -2 (COX-1 and COX-2, respectively). The NIEHS/NIH is using azoxymethane, which induces precancerous lesions, to study COX-dependent development of premalignant and malignant lesions in transgenic mice (knock-out for one isoform , heterozygous for COX-1 or COX-2, or heterozygous for both isoforms) (Langenbach, NIEHS/NIH).

Leukemogenesis

The *p53* model is being used to evaluate the mechanisms and molecular genetics of benzene, a ubiquitous environmental carcinogen to humans and rodents. Benzene exposure is associated with depression of blood-forming elements leading to anaplastic anemia followed by myelodysplastic syndrome and ultimately leukemia or lymphoma. Inhalation of benzene leads to a slower rate of delivery and a greater internal dose than other exposure routes. Studies are in progress for understanding the genetic events associated with benzene-induced cancer (French, NIEHS/NIH).

Prostate Cancer

Prostate cancer is the most commonly diagnosed malignancy in western men and is presenting a rapidly rising incidence in many countries. Environmental factors are now thought to play an important role in its genesis. Prostate cancer research has a critical need for experimental animal models with which to understand its occurrence and establish suitable prevention and intervention strategies. A study to assess the effects of dietary restriction on serum insulin-like growth factor-1 (IGF-1) levels and progression of prostate cancer in the TRAMP (Transgenic Adenocarcinoma Mouse Prostate) transgenic model is in its final stages and tissues are being prepared for histopathological evaluation. In a separate study, tissues from different prostate lobes of the TRAMP mouse are being prepared for microarray studies to identify genes that might explain the differential response of the different lobes to prostate cancer. Results from TRAMP will be compared with wildtype mice (Maronpot, NIEHS/NIH).

Skin Cancer

Studies at the NIEHS/NIH are investigating the role(s) of cyclooxygenases-1 and -2 (COX-1 and COX-2, respectively) in ultraviolet (UV)-induced skin cancer using transgenic mouse models. Studies are comparing effects of each transgenic strain to wildtype mice. Mice depficient in COX-2, but not COX-1, exhibit dose-dependent increases in UV-induced epithelial skin damage and cell death compared with wildtype mice (Langenbach, NIEHS/NIH).

Comparative Carcinogenesis

Leiomyomas

Uterine leiomyomas or "fibroids" are benign tumors clinically diagnosed in 20-30% of U.S. women during their third to fourth decade of life. Uterine leiomyomas pose a major public health cost in terms of outpatient care and hospital costs for surgical procedures (hysterectomies). Research is ongoing at the NIEHS/NIH to delineate some of the basic biological and molecular processes important in uterine fibroid development and growth that might be applied to the development of alternative treatment regimens. Archival mouse and human tissues are being used to determine the presence of growth factors in uterine leiomyomas and leiomyosarcomas. These studies have focused on evaluating the role of cell proliferation or prolonged cell survival in the growth of uterine leiomyomas, and other studies are underway to determine the role of modulators of apoptosis such as BCl-x and Mcl-1. Preliminary results show that both positive and negative regulatory proteins of apoptosis are present in female uterine leiomyomas and apoptosis does not appear to have a significant role in tumor development (Dixon, NIEHS/NIH).

Ovarian Cancer and Dysfunction

Epidemiological and experimental data provide support for various exposures, including occupational, environmental, and therapeutic, that can impact development of reproductive cancers. Ovarian cancer is the leading cause of death from gynecological cancers and uterine leiomyomata (fibroids) are the most common gynecological tumor. Disruption of ovarian function greatly impacts the reproductive, endocrine, and ultimately general health of women. NIEHS/NIH studies are focusing on identifying the ovarian target cell(s) and biochemical and molecular mechanisms by which synthetic or naturally-occurring environmental chemicals cause ovarian cancer or dysfunction, determining key genes and signaling molecules involved in disease etiology, and exploring how modifications in these pathways may ameliorate these disorders. Ovarian toxicants, such as phthalates and glycol ethers, and ovarian carcinogens, such as dioxins and furans, are being studied. Current efforts are also defining the role of genes and signaling molecules in ovarian, breast, and uterine function and disease (Davis, NIEHS/NIH).

Imaging Technologies

Magnetic resonance imaging (MRI) techniques offer great promise as a non-invasive method for following the progression and regression of toxic and carcinogenic processes in experimental animals. Numerous efforts are ongoing to establish practical applications of MRI to experimental toxicology and carcinogenesis, establish rapid data acquisition schemes for *in vivo* microscopy of laboratory animals, establish techniques for non-invasive histology on blocks of fixed tissue specimens, and develop methods for MR phenotyping of genetically modified mice with provision for Internet access to MR images. An MR mouse atlas defining the anatomy of perfusion-fixed whole mice of commonly used strains is being developed. An initial pilot study to utilize MR imaging for rat teratology studies has been completed and data are being analyzed (Maronpot, NIEHS/NIH, see also page 47).

Areas for Future Initiatives and Resources

Future initiatives in mechanistic-based carcinogenesis research will involve an integration of information from traditional and microchip array-based studies to guide the development of new models for carcinogen identification and study. The approach towards gaining insight into critical molecular changes occurring during the carcinogenic process will be two pronged, *i.e.*, examinations of gene activation and repression following acute chemical treatment, coupled with examination of the profiles of gene expression in fully developed tumors. As the NTP progresses in understanding the complex signaling pathways that are activated or repressed during carcinogenesis, the program will be able to select transgenic animal models that best mimic processes occurring in human tissues, providing a firm foundation for cross species extrapolations of hazard. The NIEHS/NIH is currently determining the utility of transgenic models for carcinogen identification and assessing how they might be used in the NTP's testing strategy to complement and/or replace the traditional models. The NIEHS/NIH plans to develop a draft strategy and seek external input from its advisory Board, scientific community, regulatory agencies, and the public. The NIEHS/NIH also plans to participate in an initiative currently ongoing at the National Cancer Institute/NIH to evaluate approximately 50 existing transgenic or knockout models of cancer-related genes for their more immediate application to hazard identification studies.

RISK ASSESSMENT EVALUATIONS

Health, research, and regulatory agencies make decisions regarding the protection of public health based on scientific information from a variety of sources. In the evaluations of human risk, several areas of uncertainty often exist:

- adequacy of animal models to detect toxicologically-induced disease end points,
- adequacy of animal models to accurately reflect human risk,
- adequacy of mathematical models used to extrapolate high dose effects to environmental or occupational exposure levels,
- adequacy of information about human exposures to environmental toxicants, and
- adequacy of information about inter-individual variability and sensitive sub-populations.

The NTP's effort in risk assessment is closely tied to its growing initiatives in mechanism-based toxicology and carcinogenesis. This linkage provides opportunities to improve priority setting, use mechanistic response relationships in the "low dose" range, select the most appropriate experimental systems for estimating risk, and develop scientifically based models for specific subpopulations (*e.g.*, age, gender, genetic predisposition, ethnicity, etc.). Increased knowledge about the mechanisms responsible for environmentally induced disease coupled with the development of sensitive and specific biomarkers of exposure and tests for biological effect are important in detecting and monitoring the early insult(s) of environmental toxicants and evaluating those effects under low dose exposures. All of these components can contribute scientific data about potential toxicity and carcinogenicity of environmental agents and strengthen the science base for risk assessment.

EPIDEMIOLOGY

Current Research Initiatives

The presence of toxicants in the environment is a potential threat to human health and the extent of that threat is unclear. Environmental toxicants may produce a variety of health effects depending upon the timing of exposure, dose, individual susceptibility, and other, yet unidentified, factors. Many of the studies currently underway or planned by the NTP investigate occupational or environmental exposure to toxicants as potential risk factors for specific health effects. Table 17 lists some specific exposures and health effects presently under consideration.

In the past, environmental research often has studied the crudest, most easily measurable health effects (cancer or death). More subtle damage (*e.g.*, infertility, neurological function, or endocrine imbalance) is often harder to detect although such effects may be more common and impact a greater number of individuals. Increasingly, the NTP looks for ways to improve its ability to detect potential health effects from environmental exposures. Efforts are underway by the NTP to develop sensitive techniques for measuring the phenotypic effects of exposure and studying genetic changes associated with disease etiology, identify new genes involved in response to environmental toxicants, and identify genetic polymorphisms. Some of the important tools in this effort come from recent advances in biotechnology that include more sensitive methods for measuring low dose exposures, detecting early stages of disease, determining genetic susceptibility, and evaluating illnesses for which the causes are largely unknown but environmental etiology is plausible (*e.g.*, neurologic disease).

In addition, the development of novel statistical tools is aiding epidemiologic investigations. Such tools are being applied to evaluations of gene-environment interactions and genetic susceptibility. Improvements in study designs and associated techniques for data analysis are facilitating the study of interactions between genetic susceptibility factos and environmental exposures (Umbach, NIEHS/NIH).

Table 17. Agents under Consideration as Risk Factors for Specific Human Health Effects

Exposure	Health Effect
Air pollution	Childhood respiratory diseases
Beryllium	Chronic beryllium disease
Diet	Nonmalignant respiratory disease in adults, lung cancer
Ionizing radiation	Female reproductive health and outcomes
Lead	Amyotrophic Lateral Sclerosis and reproductive disorders
Magnetic fields	Breast cancer
Mercury	Amyotrophic Lateral Sclerosis
Nickel	Reproductive effects in females
Organochlorines (DDT, PCBs, dioxin, PCDFs)	Birth term and size, childhood neurologic deficits, child development, Type 2 diabetes, adolescent body habitus, breast cancer, thyroid function, and reproductive disorders
Pesticides (insecticides, herbicides, fungicides, and fumigants)	Childhood diabetes, lupus, macular degeneration, neurobehavioral function, Amyotrophic Lateral Sclerosis, adult neurologic deficits, attention deficit hyperactivity disorder, Parkinson's Disease, primary intracranial gliomas, and reproductive health effects
Radon	Lung cancer and childhood leukemia
Ionizing radiation	Reproductive disorders
Silica	Systemic lupus erythematosis
Smoking and environmental tobacco smoke	Nonmalignant respiratory disease in adults, asthma, chronic bronchitis, lung cancer
Soy	Child development
Uranium mining	Lung and other cancers

Gene/Environment Interactions

Humans can vary widely in how they respond to environmental exposures. Genetic susceptibility may play an important role in many aspects of environmental carcinogenesis as well as non-cancer diseases. The NIEHS/NIH is interested in the discovery of functionally important genetic polymorphisms for genes that either modify an individual's response to an environmental toxicant or are part of the pathway(s) responding to that exposure. In addition, they are addressing how genetic susceptibility to carcinogens may differ by age, ethnicity, sex, or lifestyle factors (*e.g.,* smoking, alcohol consumption) in order to better integrate environmental and genetic factors in understanding human disease etiology.

Initial efforts at the NIEHS/NIH have focused on disease susceptibility and DNA repair gene polymorphisms and on the measurement of repair capacity. Researchers have demonstrated that polymorphisms in carcinogen metabolism genes and DNA repair genes affect the risk of bladder cancer and found evidence for gene-environment and gene-gene interactions. They have also demonstrated that polymorphisms in DNA repair, vitamin D receptor, and hormone metabolism genes affect the risk of prostate cancer. They are continuing their efforts to investigate relationships between common polymorphisms in DNA repair genes and risk of disease and are adapting functional assays to measure a person's DNA repair capacity for application to epidemiology studies (Taylor, NIEHS/NIH).

In collaborative efforts with other groups, including the NCI/NIH, University of Occupational and Environmental Health in Japan, and several U.S. universities, the NIEHS/NIH is testing the impact of a group of cancer susceptibility genes in case-control studies of cancer of the bladder, skin, lung, live, colon, stomach, prostate, and breast. They are also exploring whether ethnic difference in cancer incidence might be due to genetic differences in addition to differences in exposure. This information should improve our understanding of the basis of genetic risk and the accuracy of environmental risk assessment for environmental toxicants.

The NIEHS/NIH is also working to develop methods for identifying unique biomarkers for whole genomes that can be used to test hypotheses concerning the role of environmental and genetic factors in disease etiology (Bell, NIEHS/NIH).

Scientists at the NIEHS/NIH are also testing the hypothesis that environmental exposures produce specific patterns of gene mutation in human tumors. If correct, such patterns could be used both to identify novel critical target genes and to suggest mutational mechanisms by which an environmental agent causes disease. In addition, they could be powerful screening tools in studies of environmental risk and for use in early prevention and early diagnosis. They have examined exposure-specific mutations of *ras* and *p53* in epidemiology studies of 1) aflatoxin and hepatitis B virus in hepatocellular carcinoma, 2) smoking, occupation, and metabolism gene polymorphisms in bladder cancer, 3) organochlorines and diabetes in pancreatic cancer. In a related effort they are investigating whether exposure correlates with the pattern of mutation in premalignant and normal lung tissues and whether such mutations may have prognostic significance for lung cancer development. A prospective study is underway of people at high risk of developing lung cancer to test if molecular changes in normal and preneoplastic bronchial epithelium are correlated with exposure or neoplastic progression. This will be the first systematic, prospective study that follows the natural development of lung cancer precursor lesions and carcinoma *in situ* over time with sequential biopsies; the current practice is to follow them without intervention (Taylor, NIEHS/NIH).

NCTR/FDA scientists are conducting research to provide new knowledge on the identification of subpopulations that are not only more susceptible to chemical carcinogens, but also those that are likely to experience adverse drug reactions or decreased therapeutic drug efficacy. This research should 1) facilitate a better understanding of the mechanisms of human carcinogenesis, 2) provide an estimation of human exposure to direct and indirect-acting carcinogens, 3) assess the importance of inter-individual differences in carcinogen and drug bio-activation, detoxification, or induced changes in gene expression, and 4) suggest intervention strategies for human cancer prevention. Projects studying the etiology of human cancers of the colon/rectum, pancreas, larynx, breast, ovary, prostate, prostate, lung, urinary bladder, bone marrow, and esophagus are ongoing (Kadlubar, NCTR/FDA).

Immune Function

There is increased interest at the NIEHS/NIH in identifying environmental factors that might impact the immune system and development of autoimmune disease. The Carolina Lupus Study is a population-based case control study of patients recently diagnosed with systemic lupus erythematosus, an autoimmune disease that severely damages the kidneys, joints, and other tissues, or other autoimmune disease living in eastern North Carolina or South Carolina. It focuses on measurement of endogenous hormone exposure, exogenous sources of estrogen, occupational exposures (silica), and medical history-related factors (Cooper, NIEHS/NIH).

Leukemia

In a previous study the NIEHS/NIH explored the relationship between environmental exposures and risk for acute leukemia in adults. The study evaluated risk factors for specific subtypes of leukemia defined by chromosomal aberrations and molecular changes. The results suggest that genetic or familial factors may help to define groups who are susceptible to developing leukemia following environmental insult. They are now examining the link between a family's cancer history and disease risk to determine if it can serve as an indicator of enhanced susceptibility due to increased likelihood of having one or more of the several gene polymorphisms that are involved in cancer etiology (Sandler, NIEHS/NIH).

Lung Disease

The NIEHS/NIH is exploring the etiologic role of genetic susceptibility for lung cancer through investigations of several diverse populations: African-Americans, U.S. Caucasians, and Chinese (London, NIEHS/NIH). Another study is examining cancer risk in Czech uranium miners and investigating their cancer incidence using linkage with a population-based cancer registry (Sandler, NIEHS/NIH). The relationship between residential radon exposure and risk for lung cancer and/or childhood leukemia is also being studied (Sandler, NIEHS/NIH).

Both the NIEHS/NIH and NIOSH/CDC are interested in identifying factors that might increase risk for non-cancer lung diseases. A NIEHS/NIH study is examining the role of genetic factors, diet, and environmental exposures (including air pollution, smoking and environmental tobacco smoke) relative to risk for non-malignant respiratory disease (asthma, chronic bronchitis) using a cohort of over 50,000 older adults of Chinese ethnicity in Singapore (London, NIEHS/NIH). Asthma is the most common chronic disease in children. Another project is addressing childhood respiratory disease globally by examining several populations with varying prevalence of asthma: Southern California, Mexico City, and Wuhan, China (London, NIEHS/NIH). Currently a multi-facetted study is being conducted by NIOSH/CDC to evaluate potential environmental and genetic determinants for chronic beryllium disease (Weston, NIOSH/CDC).

Neurologic Disease and Function

Several investigations are examining the role of environmental toxicants on neurologic disorders. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting the motor neurons of the brain stem and spinal cord for which there is evidence of possible environmental etiology. A current case-control study at the NIEHS/NIH is examining the effects of cumulative lifetime exposure to lead and other neurotoxins including mercury, solvents, and pesticides on risk for ALS (Kamel, NIEHS/NIH).

Several projects are targeting agricultural workers and the potential health effects of pesticide exposures. In the early 1990s, the NCI/NIH initiated a prospective study of cancer incidence in a cohort of licensed pesticide applicators and their spouses who have substantial direct and indirect exposure to pesticides, the Agricultural Health Study. The NIEHS/NIH has joined this effort to examine non-cancer-related health effects (Sandler, NIEHS/NIH). A case-control study within the Agricultural Health Study is examining whether pesticide exposure is associated with an increased risk for Parkinson's Disease. Another study is examining the effects of occupational pesticide exposure in farm workers of central Florida to elucidate the effects of pesticide exposure on neurologic function and other health problems (Kamel, NIEHS/NIH). A NIOSH/CDC initiative is investigating what environmental factors might increase susceptibility of rural residents for primary intracranial gliomas (brain cancer) and evaluating the impact of genetic polymorphisms with their associated, relevant exposures on susceptibility to gliomas (Ruder, NIOSH/CDC).

Selected organophosphate pesticides pose known or suspected risk to health including behavioral and nervous system effects. A project is working to improve methods used to assess neurobehavioral effects of organophosphate pesticides. This project is collaborative between NIOSH/CDC and the Ohio State University and the Oregon Health Sciences University. Preliminary findings suggest that objective assessment methods may be critical for uncovering subtle neurobehavioral changes following pesticide exposures (Waters, NIOSH/CDC).

The NIEHS/NIH is also focusing efforts on children's neurologic health and environmental exposures. Data collection continues for one of the first population-based studies of attention-deficit hyperactivity disorder. This is part of an effort to describe environmental risk factors for this common childhood ailment (Sandler, NIEHS/NIH).

Reproduction and Development

An area of NTP interest is the potential impact of environmental toxicants on reproduction and development. Epidemiological efforts at the NIEHS/NIH are targeted toward describing the basic biology of human reproduction, developing improved epidemiological tools for detecting environmental damage, and identifying environmental factors that might interfere with human reproduction. An initiative is ongoing to develop methods that will allow identification of environmental hazards affecting fertility, ovarian function, and pregnancy (Baird, NIEHS/NIH). The NIEHS/NIH is creating a specimen repository that can be used to evaluate environmental exposures and health effects in pregnant women and their unborn children (Hoppin, NIEHS/NIH).

The NIEHS/NIH is conducting a population case-control study of facial clefts in Norway to determine the impact of these birth defects on survival and reproduction, as well as the risk of birth defects in the offspring of affected parents (Wilcox, NIEHS/NIH).

The NIEHS/NIH is studying the worldwide consequences for child development from exposure to polychlorinated biphenyls (PCBs) and similar compounds. Since 1979 they have followed a group of 117 children in Taiwan who had transplacental exposure to PCBs and polychlorinated dibenzofurans (PCDFs) due to a food poisoning incident. They've also studied adults who were poisoned, some of whom are mothers of the children, for possible reproductive disorders. Recent results show that the reported behavioral abnormalities in the children are diminishing although their developmental delay is fixed. Only minimal effects on menstrual flow in the mothers have been observed with no effects on fertility, family size, or libido (Rogan, NIEHS/NIH). Another project is examining health effects associated with *in utero* exposure to persistent halogenated organic pollutants. They are interested in knowing why African-Americans, but not whites, would be at an increased risk from PCBs (Longnecker, NIEHS/NIH).

Approximately 15% of infant formula sold in the United States is soy-based. Soy formula contains large amounts of isoflavones (genistein and daidzein). The NIEHS/NIH is undertaking a clinical trial to determine if exposure to soy estrogens prolongs anatomical and biochemical evidence of estrogen exposure and response (Rogan, NIEHS/NIH).

Women's Health

Several efforts are underway at the NIOSH/CDC to examine potential effects of occupational exposures on women's health. One is to develop biological markers of female reproductive health and apply them to evaluations of occupationally exposed women. Reproductive endocrine biomarkers will be assessed in Russian workers at a nickel refinery and Cree Native Americans exposed to polychlorinated biphenyls, DDT, and lead (Kesner, NIOSH/CDC). Another study is examining the effects of ionizing radiation and circadian rhythm disruption on reproductive health of female flight attendants (Whelan, NIOSH/CDC).

Women's reproductive health is the focus of initiatives at both the NIEHS/NIH and NIOSH/CDC. As part of the Agricultural Health Study, the NIEHS/NIH is examining whether pesticide and other farm exposures affect menstrual cycle abnormalities, fetal loss, and preterm births among women living on farms (Sandler, NIEHS/NIH). Another study is relating environmental exposure to clinical outcomes following *in vitro* fertilization (Weinberg, NIEHS/NIH).

Breast Cancer

In the United States, breast cancer accounts for about 30% of all new cancer cases among women and known risk factors account for only about half of breast cancer risk. The etiology of breast cancer is complex and both genetic and environmental factors are important. The NIEHS/NIH is planning to study the potential genetic and environmental risk factors for breast cancer in a cohort of sisters of women with breast cancer. While the focus of the Sister Study is on the currently disease-free sisters who may later develop disease, a companion study will include women who were recently diagnosed, provided they have a disease-free sister who also enrolls (Sandler, NIEHS/NIH).

The hypothesis that magnetic fields influence breast cancer risk is based upon reported effects of magnetic fields on melatonin and some limited epidemiological evidence. The NIEHS/NIH is investigating whether residential magnetic fields, as assessed by wiring configuration and direct measurements, are associated with increased risk for breast cancer. A second project is examining whether specific combinations of alternating current and direct current fields increase that risk. This study is targeting Latino and African-American women in an older urban area where magnetic field exposures are higher than in newer suburban areas (London, NIEHS/NIH).

NIOSH/CDC is investigating breast cancer incidence among female workers exposed to polychlorinated biphenyls (PCBs). These compounds are suspected breast carcinogens because of their estrogenic and lipophilic properties (Whelan, NIOSH/CDC).

Researchers at the NCTR/FDA and the FDA's Office of Women's Health are collaborating on a study of breast cancer in African-American women that is examining metabolic modification of dietary and hormonal factors. The study is a post-market surveillance of chemical toxicants found in foods, drugs, cosmetics, and medical devices and their relationship to human breast cancer risk (Kadlubar, NCTR/FDA).

Areas for Future Initiatives and Resources

Molecular Epidemology

Molecular epidemiology studies will be progressively more important to the NTP. For example, such studies will be used increasingly in the Report on Carcinogens (see page 81) to support listing agents as known human carcinogens. It is therefore of paramount importance to develop principles for evaluating and interpreting these studies. The NTP will look for input on this issue from its advisory Board, experts in this field, regulatory agencies, and the public.

Studies of Specific Agents

Analytic epidemiology studies investigating the relationship of toxicant exposure to specific health effects often rely on the fortuitous identification of exposed populations. Agents of interest to the NTP can occasionally be studied in this way. An example is methyl-tertiary-butyl ether (MTBE), which is presently used as a gasoline additive. Public concern has been generated by the potential for inhalation exposure and its presence in ground water. Animal studies show that MTBE causes hematopoietic, kidney, liver, and testicular tumors in rodents; however, little is known about its carcinogenic potential in humans. MTBE has been used clinically to treat gallstones. Researchers at the NIEHS/NIH are presently investigating whether a cohort of MTBE-treated patients can be identified, and if so, it will be followed for cancer incidence or mortality by linking with the Surveillance Epidemiology and End Results (SEER) Cancer Registries or the National Death Index.

EXPOSURE ASSESSMENT

The NTP recognizes that accurate and complete exposure assessment is critical both to the success of epidemiology studies of toxicant exposure and to the utility of such studies for risk assessment and public health policy. However, the availability of human data is often the weakest component of risk assessment and limits the effective utilization of experimental data for making decisions about chemical exposures. Increased knowledge about the mechanisms that are responsible for environmentally induced diseases coupled with both sensitive and specific biomarkers and tests of biological effect from exposure are important in detecting and monitoring the early insult(s) of environmental toxicants and in evaluating those effects under low-dose exposure.

Current Research Initiatives

Exposure Assessment Surveys

Advances in analytical methodologies now enable the detection of environmental and occupational chemicals in small biological samples (*e.g.*, blood, urine, and hair). Taking advantage of such advances, the NTP is leading a new interagency human exposure assessment initiative in collaboration with NCEH/CDC, NIOSH/CDC, and EPA to quantify the body burdens of chemicals released into the environment and workplace. Discussions are underway about the scope and feasibility of such an effort.

The NIEHS/NIH and NCEH/CDC are collaborating on a pilot project for quantifying approximately 70 chemicals found in either human blood or urine that are considered to be endocrine disruptors. Information about this project is given on page 21.

NIOSH/CDC is currently planning a project focusing specifically on identifying workers exposed to reproductive toxicants (Moorman, NIOSH/CDC, see page 24).

The NIEHS/NIH through the National Allergen Survey is assessing the allergen types and levels in the nation's housing to provide estimates of allergen exposure in the U.S. population. Information from this survey should facilitate future studies examining exposure/asthma prevalence/morbidity relationships and evaluation of regional, ethnic, socioeconomic, and housing characteristic differences in the allergen burden. A clinical trial has begun to determine if environmental intervention aimed at reducing indoor allergen levels in homes can prevent sensitization and decrease asthma prevalence in high risk children (Zeldin, NIEHS/NIH).

Exposure Assessment Monitoring

The NIOSH/CDC has several ongoing projects aimed at monitoring the health of workers and determining whether methods for reducing exposure are effective. Based upon animal toxicology data, pesticides have potential effects on neurologic function, skin reaction, and may be carcinogens. The EPA has granted cut-rose producers an exemption to its Worker Protection Standard that excludes workers from entering pesticide-treated areas during restricted entry intervals. Workers are allowed entry to harvest roses for up to three hours per day. At the request of the EPA, NIOSH/CDC is conducting a study to determine if early contact with pesticide-treated roses poses a health risk to rose harvesters. Researchers will evaluate pesticide exposures of workers in rose-growing greenhouses and the effectiveness of worker practices and personal protective equipment worn to reduce exposures (Sanderson, NIOSH/CDC).

Through an interagency agreement with the NIEHS/NIH, a major effort is underway at the NIOSH/CDC to better characterize worker exposure by obtaining “real world” information about worker practices, exposures, and possibly related health effects. Initiatives currently underway target asphalt fumes and 1-bromopropane (see page 23).

Through an interagency agreement with the NIEHS/NIH, NIOSH/CDC has established and validated a testing battery for use in assessing alterations in immune fuction in individuals exposed to potentially hazardous compounds in the workplace. Workers have been screend for allergy to natural rubber latex. These studies are attempting to address the importance of respiratory versus dermal sensitization in development of latex allergy and evaluate the effectiveness of measures initiated for prevention of occupational latex allergy (Germolec, NIEHS/NIH; Munson, NIOSH/CDC).

Natural rubber latex (NRL) allergy is an important cause of occupationally related allergy and asthma in health care workers. Work at NIOSH/CDC has focused on development and application of immunologic assays for assessing workplace exposure to NRL. This project is evaluating NRL-specific serum IgG as a biomarker of exposure in serum of health care workers. These methods should have application for assessing persistence of NRL allergen in health care environments, including the efficacy of decontamination procedures (Weissman, NIOSH/CDC).

Exposure Assessment Biomarker and Methods Development

Exposure of workers to a chemical results in appearance of the chemical or its metabolite(s) in the workers’ blood, breath, urine, or other biological medium. Biological monitoring is complementary to workplace environmental monitoring and useful for documenting occupational exposure situations where dermal absorption or ingestion is a significant route of uptake. The NIOSH/CDC has developed biological monitoring analytical methods for a variety of agents. Many of these biomarkers address biological monitoring of genotoxicity, protein adduct formation, and genetic polymorphisms as well as internal exposure monitoring of biological specimens (*e.g.*, blood, urine, tissues). Such biomarkers should have application for human field studies. Urinary biomarkers have been developed for diazanon, hexane, asphalt fumes, 2-(2-methoxyethyoxy)ethanol, butoxyethanol, and 1-bromopropane (Cheever/Snawder/DeBord, NIOSH/CDC). Future efforts will include development and application of a method for biological monitoring for urinary detection of mercapturic acid conjugates for the herbicides alachlor, metolachlor, acetochlor, atrazine, and cyanazine (Snawder/DeBord, NIOSH/CDC).

Several efforts are aimed toward development of technologies to improve exposure assessment. A new generation of high performance quadrupole time-of-flight mass spectrometry (Q-TOF MS) coupled to nanoflow liquid chromatography is being developed to characterize DNA/protein adducts induced by *in vivo* exposure to asphalt fumes. This should allow macromolecular identification and characterization of adduct formation both qualitatively and quantitatively because it is more sensitive and accurate than previously used methods (Wang, NIOSH/CDC).

Some initiatives target improvements in urinary biological monitoring technology. One project is testing a novel technique, Fluorescence Microbead Immunosorbent Assay (FMIA) as a viable tool for multianalyte biological monitoring that allows simultaneous analysis of multiple pesticide analytes and cytokines in urine. Assays for acetochlor, alachlor, atrazine, glyphosate and metolachlor mercapturate are ongoing (Biagini, NIOSH/CDC). The use of insecticides and herbicides with known acute and chronic toxicities presents a significant exposure risk for agricultural workers. In efforts to minimize risk, urinary biological monitoring is an essential tool for assessing exposure, since much of it occurs through skin rather than from inhalation. New enzyme-linked immunosorbent assays (ELISA), which measure metabolites of pesticides in urine, have been developed. These and other new analytical methods are being applied to the analysis of samples from three field studies, the Farm and Family Field Study, Rose Growers Study, and Orchardists Study (Striley, NIOSH/CDC).

The NIOSH/CDC is addressing concerns about occupational allergies. Occupational exposure to organic substances can result in the development of immunologically mediated hypersensitivity reactions that may affect the upper airways (allergic rhinitis) or lower airways (asthma and hypersensitivity pneumonia). Researchers are working to develop and validate immunoassays that can be used to detect biomarkers of exposure and/or early markers of occupational respiratory diseases and monitor airborne levels of antigenic substances. The goal is to develop procedures applicable for field investigations and epidemiology studies. Current efforts are targeting occupational respiratory diseases associated with exposure to metal working fluids and microbial agents present in those fluids (Lewis, NIOSH/CDC).

Efforts are underway at the NIOSH/CDC to implement state-of-the-art methods for assessing male reproductive health in field investigations. Male reproductive hazards appear to target at least one of four major sites (endocrine system, the testes, the accessory glands, and sexual function). These methods have been field-tested in bicycling policemen and are currently being used in a study of men working in a nickel refinery in Monchegorsk, Russia, where the primary exposures of concern are nickel and cobalt. This study is being conducted in partnership with McMaster University of Ontario in Canada, the University of Tromsø in Norway, and the Kola Research Laboratory of Occupational Health in Kirovsk, Russia (Schrader, NIOSH/CDC).

Areas for Future initiatives and Resources

Exposure Assessment Methods

A great need exists for simple and low-cost exposure assessment techniques that could be used in population-based studies of disease etiology. Both laboratory-based and questionnaire methods are essential. The NTP is considering several approaches to develop such techniques. First, the program will look for opportunities to interface with existing or planned exposure initiatives, such as National Health Exposure Assessment Survey (NHEXAS) or the Interagency Human Exposure Assessment Initiative currently being developed by the Center for Environmental and Nutritional Research. As available, the NTP will use data being collected by these projects to develop and validate exposure assessment techniques. Second, the NTP will interface with existing or planned health surveys such as the National Health and Nutrition Examination Survey (NHANES) to encourage the incorporation of occupational histories and other exposure measures into such surveys. Analysis of the resulting data could also be useful for developing and validating exposure assessment techniques. Finally, the NTP will continue to review ongoing laboratory research within its program and determine whether this might be applicable to the development of biomarkers useful for population-based studies.

Surveys of Occupational Cohorts

There are many agents where carcinogenicity has been demonstrated in animal studies but no published studies exist of cancer in humans. The NTP is interested in whether it would be possible to fill this data gap by surveying occupationally exposed populations and is moving forward with a strategy to address this issue. The first step will be to use the NIOSH National Occupational Exposure Survey (NOES) to identify workers who are exposed to specific agents and to determine how many workers are exposed and in what industries. This information will then be used together with the Report on Carcinogens to identify and prioritize agents for further study. Mortality studies for selected agents could then be conducted by linking workers previously employed in specific industries to the Surveillance Epidemiology and End Results (SEER) Cancer Registries or the National Death Index. Initial studies could use ever employment in an industry as a marker of exposure; however, more detailed subsequent studies could use job titles and duration of employment to make more specific inferences about the relationship of exposure to cancer mortality. Although the preliminary surveys will be relatively straightforward, the mortality studies will be complex and require access to industry records. Collaboration with NIOSH/CDC is a potential mechanism for gaining access to industry records.

TOXICOKINETIC AND BIOCHEMICAL MODELING

Current Research Initiatives

Risk assessment involves using factual data to determine the plausibility and magnitude of health effects for individuals and populations from exposure to hazardous agents. Useful to risk assessment is the development of biologically based models for estimating human risk. These models are mathematical representations of physiological and biochemical processes that occur in laboratory animals and humans. These models can provide a scientifically sound basis for evaluating data in animals and then extrapolating that information across species to determine if and how exposure to an agent might cause health effects in humans.

The process of developing biologically based models is iterative. It relies upon first developing a simple model based upon available data, testing predictions of the model experimentally, and then making adjustments or expanding the model's complexity as more data become available from studies in cell cultures, animals, and humans.

Toxicokinetic Modeling

Complete dosimetry of a chemical or physical agent describes its absorption, distribution, metabolism, and elimination (ADME) at differing levels of exposure, over all ages, via multiple routes of exposure, and under varying genetic backgrounds in humans and test animals. Data from NTP chemical disposition, metabolism, toxicokinetic studies (see page 27) are used in these efforts. In recent years, NTP has expanded its efforts in toxicokinetics to provide a better understanding of the behavior of chemicals under study in test animal species.

An ongoing initiative at the NIEHS/NIH is to integrate data from a number of levels to address knowledge gaps that create uncertainty in risk assessment for receptor-mediated toxicants. These studies focus on dioxin and its structural analogs as a prototypical receptor-mediated toxicant. Dioxin-like compounds are ubiquitous environmental contaminants and their persistence in the environment, their lipophilicity, and subsequent bioaccumulation through the food chain result in chronic human exposure. Dioxin has been classified as a known human carcinogen; however, considerable controversy exists over the potential human health risk posed by daily exposure. Data from animal models, cell systems, and human studies are being used to develop risk assessment models for dioxin. These studies will attempt to identify sensitive subpopulations and develop strategies for replacing default methods for estimating the range of expected risks in the population (Walker, NIEHS/NIH).

Biochemical Modeling

For many chemicals or classes of chemicals, there is emerging data related to gene expression, protein levels, receptor binding and interaction, and cellular protein changes that should allow development of biochemical models more complex than simple ADME models. Such models can provide mechanistic insights into the origin of biological changes at the cellular and molecular levels resulting from a particular exposure and improve risk assessment. Research is underway to develop computational methods to provide detailed atomic level descriptions of biomolecules such as protein-DNA complexes (Darden, NIEHS/NIH). At the NIEHS/NIH mechanistic models are being constructed to characterize Ah receptor-dependent transcriptional activation of dioxin-responsive genes and enzyme induction in 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) treated rats (Portier, NIEHS/NIH; Walker, NIEHS/NIH). Future efforts will address the development of a prostate growth and development model to evaluate the potential role of environmental agents in prostate carcinogenesis through their interference in endocrine signaling pathways.

Physiologically Based Pharmacokinetic Models

Physiologically based pharmacokinetic (PBPK) models have an improved and realistic description of key physiological processes and biochemical activities that affect both ADME of the parent compound and its metabolites. Substitution of human physiological and biochemical parameter estimates into models characterized for laboratory animals provides a sound scientific basis for extrapolations of tissue dosimetry across species, extrapolation from high exposures to low exposures, and extrapolation across different routes of exposure. Because PBPK models use parameters that are measurable in human populations, these models can also be used to evaluate the impact of inter-individual variability.

PBPK models have been created or are under development at the NIEHS/NIH to evaluate exposure-response relationships for carcinogenicity and developmental and reproductive toxicities (Table 18). Inclusion of PBPK models in the NTP Technical Reports is becoming routine. This information should aid regulatory agencies in their assessments of the potential for human risk from exposure to environmental agents (Portier, NIEHS/NIH).

Table 18. Physiologically Based Toxicokinetic Modeling Ongoing by the NTP

Chemical	Exposure Route	NTP Technical Report Series No.
2,3,7,8-Tetrachloro-dibenzo-*p*-dioxin[1]	oral and Topical	
Anthraquinone	oral – feed	494
Butadiene[1]	Inhalation	
Isoprene	inhalation	486
Melatonin[1]	Endogenous	
Mercury (pregnant rat)[1]	inhalation	
Methyleugenol	oral – gavage	491
Naphthalene	inhalation	500
p,p'-Dichlorodiphenylsulfone	oral – feed	501
Polychlorinated Biphenyls (209 congeners)[1]	multiple	
Primidone	oral – feed	476
Sodium Nitrite	oral – drinking water	495

[1] Ongoing PBPK modeling research

The NIEHS/NIH has also applied the use of PBPK modeling for the prediction of tissue and organ concentrations of drugs. A model has been developed for 3'-azido-3'-deoxythymidine (AZT, Zidovudine) disposition in humans and could be used to develop individualized exposure regimens to obtain specific tissue concentrations for an individual (Portier, NIEHS/NIH).

Areas for Future Initiatives and Resources

Toxicokinetic Modeling

Researchers at the NIEHS/NIH are interested in developing a library of prototypical submodels of physiological and biochemical processes (*e.g.*, gastrointestinal tract absorption, dermal absorption, placental transfer, urinary elimination, glutathione depletion and re-synthesis). Improvements in toxicokinetic modeling are also needed. The NTP would like to improve the reliability of toxicokinetic models with greater accounting for mass balance, direct information on the enzymatic kinetics of the metabolic elimination of the parent compound and its primary metabolites, and tissue time-course data of parent compound and its metabolites. The NTP is interested in the development of mechanistically based dose-response models for non-cancer end points in order to improve the ability to estimate human risk from environmental toxicants. One proposed effort is to characterize the disposition of environmental agents in dams and embryos during organogenesis and perinatal development. The NTP's initiatives studying *in utero* exposures (see page 46) will contribute to these efforts.

Biochemical Modeling

Researchers at the NIEHS/NIH are interested in extending their current efforts in modeling tissue responses to active toxicants (*e.g.*, TCDD) by investigating alterations in gene expression. As molecular information becomes available, efforts will be made to characterize dose-response relationships for DNA damage induced by epoxide and epoxide-forming chemicals (*e.g.*, 1,3-butadiene, isoprene, chloroprene, ethylene/ethylene oxide, propylene/propylene oxide, and styrene/styrene oxide).

The NTP is interested in testing mechanistic hypotheses by biomathematical modeling of intracellular responses to toxicant exposures (*e.g.*, relationships among peroxisome proliferator activated receptor-mediated gene expression, peroxisome proliferation, and liver tumor induction). Future NIEHS/NIH research will target this effort.

ALTERNATIVE TEST SYSTEM DEVELOPMENT AND VALIDATION

A large number of chemicals (>80,000) are currently in use. The NTP continually faces the task of determining how to acquire the scientific information about a substance(s) being evaluated that will best address identification of any related hazard from exposure and strengthen the science base. Implementing new strategies, which provide additional or more accurate information, can strengthen the science base on which regulatory decisions are based. Through the NTP, efforts are focused on the development and validation of new alternative test systems (sensitive, specific, rapid) for toxicological research that will reduce, replace, or refine animal use.

Model systems under development include non-mammalian species, transgenic species, genetically engineered *in vitro* cell systems, microchip array technology, and computer-based predictive toxicology models. In addition, through the NTP Center for the Validation of Alternative Toxicological Methods, a concerted and coordinated federal effort is being made to identify, validate, and promote regulatory acceptance of alternative test systems. University-based researchers are also involved in this alternative methods development and validation through the NIEHS/NIH extramural grants program.

TRANSGENIC MODELS

Transgenic Mouse Models

The conventional rodent bioassay has been used for over three decades and is accorded credibility in identifying carcinogens thought to pose risks for human health. An ongoing goal of the NTP is to seek other model systems for toxicology and carcinogenesis studies, especially those that can provide mechanistic information relative to understanding an agent's mode of action. The use of transgenic models holds promise for improving both the accuracy and efficacy of experimental assessment of the carcinogenic potential of chemicals. Genetically altered or “transgenic” mouse models carry activated oncogenes or inactivated tumor suppressor genes known to be involved in neoplastic processes both in humans and rodents. This trait may allow them to respond to carcinogens more quickly than conventional rodent strains. In addition, the neoplastic effects of agents can be observed in transgenic models within a time frame in which few, if any, spontaneous tumors would arise. The high incidences of spontaneous or background tumors, which occur most often late in the two-year rodent cancer studies, are among the most confounding factors for interpreting the findings of chemical carcinogenesis and their implications for human health. The use of target or reporter genes also allows for direct molecular and cellular analysis of a chemical’s effects in these models and can provide additional mechanistic information about mode of action.

Over the past few years, the NIEHS/NIH and NTP have been actively evaluating transgenic strains in toxicological testing strategies. Based on current evaluations, the models with greatest potential usefulness at this time are the $p53^{def}$ (*p53+/-*heterozygous) and Tg.AC (*v-Ha-ras* transgene). The Tg.AC mice carry a *v-Ha-ras* oncogene that represents a class of oncogenes that plays a key role in signal transduction pathways that regulate cell proliferation and are detectable in the early stages of tumor induction. The heterozygous $p53^{def}$ mouse lacks a member of a class of suppressor genes that has an important role in cell cycle control; loss of function is associated with progression of tumors to malignancy. These strains show specificity for being able to identify genotoxic agents ($p53^{def}$) and both genotoxic and nongenotoxic agents (Tg.AC) (Tennant, NIEHS/NIH). Studies have evaluated whether Tg.AC and p53+/- mice metabolize xenobiotics similarly to wild type mice, FVB/N and

C57Bl6, respectively, and found similar patterns of urinary metabolites when animals were treated with benzene, methacrylonitrile, or ethoxyquin. Likewise the expression patterns of cytochrome P450 enzymes, CYP2E1, CYP1A2, and CYP3A, in liver microsomal protein and glutathione reductase in liver cytosol show quantitatively similar levels to the respective wildtype strain (Burka, NIEHS/NIH).

The NTP has been involved in an international effort to evaluate the Tg.AC and $p53^{def}$ models organized by the International Life Sciences Institute and with participation by over 50 organizations representing private sector pharmaceutical and chemical companies, and government research and regulatory agencies (Tennant, NIEHS/NIH). In addition, the NIEHS/NIH is carrying out its own evaluation of the utility of transgenic mouse models for carcinogen identification. This information will be useful to the program in determining strategies for use of these models in toxicology and carcinogenesis testing (Pritchard, NIEHS/NIH).

The NIEHS/NIH is also evaluating the usefulness of other transgenic models including:

- TRAMP (Transgenic Adenocarcinoma Mouse Prostate) mice (Maronpot, NIEHS/NIH),
- p53 null mutants on an FVB/N background (Mahler, NIEHS/NIH),
- p16 transgenic mice (carries targeted deletion in *Cdnk2a* locus that eliminates expression of $p16^{INK4a}$ and $p19^{ARF}$) (Dunnick, NIEHS/NIH).
- Tg.NK mouse transgenics (contains c-*neu*, the human breast cancer oncogene homologue of *erb*B2 and develops palpable mammary tumors after 20 weeks of age) (Rao, NIEHS/NIH).
- COX 1 and COX2 knockout mice for cyclooxygenase isoforms 1 and 2, repectively) (Langenbach, NIEHS/NIH).
- Pghs mice homozygous (-/-) for prostaglandin H synthases 1 and 2 (Zeldin, NIEHS/NIH).
- Brca2 mouse models carrying one (hemizygous) or two (homozygous) mutant alleles of Brca2 exon 27 (carboxy terminal domain of *BRCA2*). Currently using fluorescent microsatellite marker-asisted breeding techniques to transfer this Brca2 exon27 mutation onto genetic bacgrounds (BALC/c and SWR) previously shown to be highly susceptible to radiation-induced mammary carcinogenesis to see whether genetic modifiers in these mouse genomes may act independently or synergistically to facilitate mammary tumorigeneiss in Brca2-deficient mice following radiation (Wiseman, NIEHS/NIH).
- Mouse lines recessive for functional ER-alpha and ER-beta (estrogen receptor) signaling systems. ERKO mice are recessive for ER-alpha, BERKO mice are recessive for ER-beta, and DERKO have neither receptor functional (Korach, NIEHS/NIH).

Transgenic Fish Models

Efforts are underway to determine the usefulness of transgenic fish as an alternate model for mice and cultured cells. Transgenic technology has been applied to the study of induced somatic mutation directly at the DNA level using PhiX174 bacteriophage as an identical marker in rodents, fish (*Fundulus heterosclitus*), and cultured cells to compare dose, adduction, and DNA repair and mutation. Initial studies comparing mice and fish exposed to the potent carcinogen, 7,12-bis-hydroxymethylbenz[a]anthracene, are encouraging. These studies suggest that identical gene indicators for specific classes of chemical-induced mutations combined with analysis of biotransformation may provide a mechanistic basis for correlations between laboratory rodents and sentinel species in polluted ecosystems. Additional studies include expanding the target sequence in the transgenic vector, investigating mutagenicity in mice and fish exposed to certain environmental mixtures, and combining this approach with developmental and endocrine disruptor end points (Burkhart, NIEHS/NIH).

NTP INTERAGENCY CENTER FOR THE EVALUATION OF ALTERNATIVE TOXICOLOGICAL METHODS

The development, validation, acceptance, and harmonization of new and revised toxicological test methods are coordinated in the Federal government through the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). ICCVAM was established in 1997 in response to the 1993 NIH Revitalization Act to reduce, refine, or replace the use of animals in research and testing. The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) was established in 1998 to collaborate with ICCVAM in the development, scientific review, validation, and achievement of regulatory acceptance of new and improved test methods applicable to the needs of Federal agencies. Dr. William Stokes is the Center Director. The ICCVAM Authorization Act of 2000 (PL 106-545) established ICCVAM as a permanent committee of the NIEHS/NIH under the NICEATM. ICCVAM consists of the heads of 15 Federal agencies (ATSDR, CPSC, Departments of Agriculture, Defense, Energy, Interior, and Transportation, EPA, FDA, NIOSH/CDC, NIH, NCI/NIH, NIEHS/NIH, National Library of Medicine, and OSHA). Dr. Leonard Schechtman, NCTR/FDA, serves as chair of ICCVAM.

The Scientific Advisory Committee for Alternative Toxicological Methods (SACATM, see page 3) was chartered on January 9, 2002, to fulfill mandates specified in the ICCVAM Authorization Act of 2000. It will provide oversight to NICEATM and the ICCVAM. The NIEHS/NIH is in the process of selecting members to the SACATM; once a slate is confirmed, an initial meeting of this committee will be arranged.

In accordance with requirements of the ICCVAM Authorization Act of 2000, the report entitled *Annual Progress Report of the Interagency Coodinating Committee on the Validation of Alternative Methods (ICCVAM)* has been prepared and submitted to the NIH Director for approval and will be made publicly available. The report describes activities carried out by the ICCVAM and the NICEATM in 2001.

ICCVAM and NICEATM work to promote the validation and regulatory acceptance of toxicological test methods that are more predictive of human and ecological effects than those currently available and to communicate with stakeholders and the public. The desired outcomes from these new methods are an improvement in agencies' abilities to assess risk and make regulatory decisions and the refinement, reduction, and replacement of animals in toxicological testing. Workshops are held, as needed, for evaluation of the adequacy of existing methods, identification of areas needing alternative methods, and evaluation of proposed validation studies. A formal, scientific review process is in place for evaluation of the validation status of proposed alternative testing methods.

Contact information: NTP Interagency Center for the Evaluation of Alternative Toxicological Methods, Dr. William Stokes, Director, NIEHS/NIH, P.O. Box 12233, MD EC-17, 111 T.W. Alexander Dr., Research Triangle Park, NC 27709, T: (919) 541-2384, e-mail: iccvam@niehs.nih.gov. NICEATM/ICCVAM web site: http://iccvam.niehs.nih.gov

Alternative Method Reviews

Information and status of the following alternative method reviews, including meeting reports and background documents, are available on the NICEATM/ICCVAM web site.

Revised Up-and-Down Procedure (revised UDP)

The EPA proposed a revised Up-and-Down Procedure ("revised UDP") as an alternate for the existing conventional LD50 test used to evaluate the acute oral toxicity of chemicals. The NICEATM/ICCVAM held a scientific peer review panel meeting in July 25, 2000, followed by a teleconference on August 21, 2001, to evaluate this procedure. The panel recommended the revised UDP as a substitute for the conventional LD50 test for hazard classification testing. This method will reduce the number of animals used for acute toxicity testing. The ICCVAM has concurred with this conclusion and will develop test recommendations and forward them to the appropriate Federal agencies for their consideration.

International Workshop on *In Vitro* Methods for Assessing Acute Systemic Toxicity

ICCVAM/NICEATM held this workshop October 17-20, 2000, in Arlington, Virginia to assess the current status of *in vitro* test methods for evaluating the acute systemic toxicity potential of chemicals and to make recommendations for validation efforts necessary for characterizing the usefulness and limitations of existing methods. The workshop experts recommended *in vitro* cytotoxicity methods as an approach that could be used to estimate starting doses for *in vivo* acute toxicity studies. The ICCVAM concurred with this conclusion and will develop test recommendations and forward them to the appropriate federal agencies for their consideration. In partnership with the EPA and the International Life Sciences Institute, the NICEATM/ICCVAM sponsored a training workshop on new alternative *in vitro* and *in vivo* methods for assessing acute oral toxicity in February 2002 at the NIH Natcher Center, Bethesda, Maryland.

In Vitro Corrosivity Methods

Three alternative *in vitro* test methods – EpiDerm™, EPISKIN™, and the Rat Skin Transcutaneous Electrical Resistance (TER) assay – were developed and subsequently have been accepted as replacement assays for traditional *in vivo* corrosivity testing in the European Union. The evaluation of these assays was part of an expedited ICCVAM review of the validation status and possible current uses of *in vitro* test methods to assess the dermal corrosivity potential of chemicals and chemical mixtures. ICCVAM recommendations for the use of these methods were published in the *Federal Register* (September 28, 2001, Vol. 66, No. 189, pages 49685-49686).

Endocrine Disruptor Screening and Testing Program

At the request of EPA, ICCVAM and NICEATM are planning an expert panel meeting May 21-22, 2002, to assess the validation status of several *in vitro* assays for use in EPA's Endocrine Screening Program. NICEATM is preparing background documents on *in vitro* estrogen receptor and androgen receptor binding and transcriptional activation assays. This information will be used for evaluating the validation status of these assays.

Areas for Future Initiatives and Resources

Development and Validation of Models

The NTP is recognized internationally for its toxicology testing using rodent bioassays; however, the program recognizes the need to expand its efforts toward understanding the mechanistic basis for toxicity. Resources will continue to be used toward the development, validation, and application of alternative models for NTP research. This includes both transgenic mouse models for use in carcinogenicity research, as well as alternative models such as cell systems and fish. Efforts to develop and evaluate transgenic animals for non-cancer end points have started.

NICEATM Activities

Working together, ICCVAM and NICEATM have been highly successful in providing an organized and productive means for coordinating activities among Federal agencies relative to the validation and regulatory acceptance of alternative toxicological test methods. The NTP is fully committed toward efforts in this area. Efforts are underway for the new advisory committee and the NICEATM and ICCVAM will continue to move forward with implementation of the ICCVAM Authorization Act of 2000.

REPORT ON CARCINOGENS

The Biennial Report on Carcinogens (RoC) is a Congressionally mandated listing of substances (i) that either are known to be human carcinogens or may reasonably be anticipated to be human carcinogens; and (ii) to which a significant number of persons residing in the United States are exposed. The Secretary, HHS delegated responsibility for its preparation to the NTP who prepares the report with assistance from other Federal health and regulatory agencies and non-government institutions. The RoC is an informational, scientific and public health document that identifies and discusses agents, substances, mixtures, or exposure circumstances that may pose a carcinogenic hazard to human health. It serves as a meaningful and useful compilation of data on 1) the carcinogenicity, genotoxicity, and biological mechanisms of the listings in humans and/or animals; 2) the potential for exposure to them, and 3) the regulations promulgated by Federal agencies to limit exposures. Dr. C.W. Jameson, NIEHS/NIH, oversees preparation of the RoC.

The nomination of chemicals, substances, mixtures, or exposure circumstances for listing in or removal from the RoC is open to all interested individuals and groups. As shown in Figure 6, the review of nominations to the report is a multi-step, formal, and open process. The scientific review of nominations involves three separate reviews (NIEHS/NTP Review Group, NTP Executive Committee Interagency Working Group, NTP Board of Scientific Counselors RoC Subcommittee) followed by review and comment by the NTP Executive Committee. Public comments are solicited multiple times during the process and are provided to each review group as available. The NTP Director receives the input from all reviews plus the public comments and makes his recommendations on the nominations to the Secretary, HHS for review and approval. Additional information about the RoC is available on the NTP web site: http://ntp-server.niehs.nih.gov.

Figure 6 Report on Carcinogens Review Process

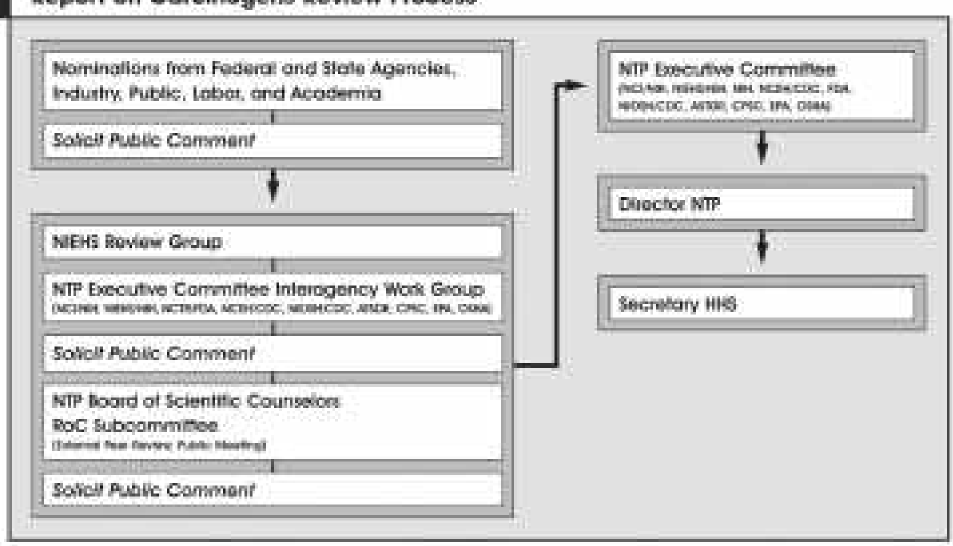


Table 19 lists the nominations completed for review for possible listing in the 10th RoC. The preparation and review process for each RoC extends over approximately a three-year period. The scientific review of nominations to the 10^{th} RoC is complete and publication is anticipated in 2002. Table 20 lists the nominations under consideration for the 11^{th} RoC. The review of these nominations is underway and publication of the 11^{th} Edition is scheduled for 2004.

Contact information: Report on Carcinogens, Dr. C.W. Jameson, Head, NIEHS/NIH, P.O. Box 12233 EC-14, 79 T.W. Alexander Dr., Research Triangle Park, NC 27709; T: (919) 541-4096; e-mail: jameson@niehs.nih.gov. RoC web site: http://ntp-server.niehs.nih.gov, see Report on Carcinogens

Table 19. Summary for Nominations Reiewed for Consideration of Listing In or Delisting from the Tenth Report on Carcinogens

Nomination (CAS No.)	Primary Uses or Exposures	Recommended for
Nominations considered in the first round of reviews		
Beryllium and Beryllium Compounds (7440-41-7)	Used in fiber optics and cellular network communications systems, aerospace, defense and other industry applications.	Updating of current listing of beryllium and certain beryllium compounds to *known to be a human carcinogen*
2,2-Bis-(bromomethyl) –1,3-propanediol (Technical Grade) 3296-90-9	Used as a fire retardant in unsaturated polyester resins, in molded products, and in rigid polyurethane foam	Listing as *reasonably anticipated to be a human carcinogen*
2,3-Dibromo-1-propanol (96-13-9)	Used as a flame retardant, as an intermediate in the preparation of the flame retardant tris(2,3-dibromopropyl) phosphate, and as an intermediate in the manufacture of pesticides and pharmaceutical preparations.	Listing as *reasonably anticipated to be a human carcinogen*
Dyes metabolized to 3,3'-Dimethylbenzidine	Dyes mainly used in textile industries with other applications in paper, plastics, and rubber industries.	Listing as *reasonably anticipated to be a human carcinogen*
Dyes metabolized to 3,3'-Dimethoxybenzidine	Dyes mainly used in textile industries with other applications in paper, plastics, and rubber industries.	Listing as *reasonably anticipated to be a human carcinogen*
IQ (2-Amino-3-methylimidazo[4,5-f]quinoline) (76180-96-6)	Found in cooked meat and fish and in cigarette smoke	Listing as *reasonably anticipated to be a human carcinogen*
Styrene-7,8-oxide (96-09-3)	Used mainly in the preparation of fragrances and in some epoxy resin formulations	Listing as *reasonably anticipated to be a human carcinogen*
Vinyl Bromide (593-60-2)	Used primarily in the manufacture of flame retardant synthetic fibers	Listing as *reasonably anticipated to be a human carcinogen* by two review groups[1] and listing as *known to be a human carcinogen* by a third review group[2]
Vinyl Fluoride (75-02-5)	Used in the production of polyvinylfluoride which is used for plastics	Listing as *reasonably anticipated to be a human carcinogen* by two review groups[1] and listing as *known to be a human carcinogen* by a third review group[2]
Nominations considered in the second round of reviews		
Broad Spectrum UV Radiation (UVR), and UVA and UVB, and UVC	Solar and artificial sources of ultraviolet radiation	Listing of UVR as *known to be a human carcinogen* Listing of UVA as *reasonably anticipated to be a human carcinogen* Listing of UVB as *reasonably anticipated to be a human carcinogen* Listing of UVC as *reasonably anticipated to be a human carcinogen*
Chloramphenicol (56-75-7)	Used widely as an antibiotic since the 1950s. Veterinary use of chloramphenicol has resulted in the occurrence of residues in animal-derived food.	Listing as *reasonably anticipated to be a human carcinogen*
Estrogens, Steroidal	Estrogens are widely used in oral contraceptives and in post-menopausal therapy for women.	Listing as *known to be a human carcinogen*

Nomination (CAS No.)	Primary Uses or Exposures	Recommended for
Methyleugenol (93-15-2)	Flavoring agent used in jellies, baked goods, nonalcoholic beverages, chewing gum, candy, and ice cream. Also used as a fragrance for many perfumes, lotions, detergents and soaps	Listing as *reasonably anticipated to be a human carcinogen*
Nickel (Metallic) and Certain Nickel Alloys	Widely used in commercial applications for over 100 years	Listing of Metallic Nickel as *reasonably anticipated to be a human carcinogen* Listing of Certain Nickel Alloys as *reasonably anticipated to be a human carcinogen* by one review group[3] and not listing in the RoC by two review groups[4]
Talc (14807-96-6) Non-Asbestiform and Asbestiform	Both forms of talc occur in various geological settings around the world. Occupational exposure occurs during mining, milling and processing. Exposure to talc non-asbestiform in the general population occurs through use of products such as cosmetics.	Review of the Talc nomination deferred pending a careful review of the literature on these materials to determine if a clear definition of the agent or agents involved in human exposures could be developed to address the concerns raised during the initial reviews over the excess lung cancers reported in people who were exposed to talc containing asbestiform fibers, or the apparent increase in ovarian cancers in women using cosmetic talc.
Trichloroethylene (79-01-6)	Trichloroethylene is widely used as a solvent, with 80-90% used worldwide for degreasing metals.	Upgrade to *known to be a human carcinogen* by one review group[3] and not to change from *reasonably anticipated* by two review groups[4]
Wood Dust	It is estimated that at least two million people are routinely exposed occupationally to wood dust worldwide. Non-occupational exposure also occurs. The highest exposures have generally been reported in wood furniture and cabinet manufacture, especially during machine sanding and similar operations	Listing as *known to be a human carcinogen*

[1] The NIEHS Review Committee for the Report on Carcinogens and the NTP Executive Committee Interagency Working Group for the Report on Carcinogens
[2] The NTP Board of Scientific Counselor Report on Carcinogens Subcommittee
[3] The NIEHS Review Committee for the Report on Carcinogens
[4] The NTP Executive Committee Interagency Working Group for the Report on Carcinogens and the NTP Board of Scientific Counselor Report on Carcinogens Subcommittee
[5] The NTP Executive Committee Interagency Working Group for the Report on Carcinogens

Table 20. Summary for Nominations Being Reviewed for Consideration of Listing In or Delisting from the Eleventh Report on Carcinogens

Nomination (CAS No.)	Primary Uses or Exposures	Basis of Nomination
1-Amino-2,4-dibromoanthraquinone (81-49-2)	1-Amino-2,4-dibromoanthraquinone is an anthraquinone-derived vat dye that is used in the textile industry.	Results of NTP Bioassay (TR-383, 1996) that reported clear evidence of carcinogenicity at multiple tumor sites in multiple species of experimental animals.
Certain Heterocyclic Amines (three nominations): 1) 2-Amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ) (77094-11-2) 2) 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) (77500-04-0) 3) 2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) (105650-23-5)	MeIQ, MeIQx, and PhIP are heterocyclic amines that are formed during heating or cooking and are found in cooked meat and fish.	IARC[1] finding of sufficient evidence of carcinogenicity of MeIQ, MeIQx and PhIP in experimental animals (Vol. 56; 1993).
Cobalt Sulfate (10124-43-3)	Cobalt sulfate is used in electroplating and electrochemical industries. It is also used as a coloring agent for ceramics, a drying agent in inks, paints, varnishes and linoleum, and has been added to animal feed as a mineral supplement.	Results of NTP Bioassay (TR-471, 1998) that reported clear evidence of carcinogenic activity in female F344/N rats and male and female B63F1 mice and some evidence of carcinogenic activity in male F344/N rats.
Diazoaminobenzene (DAAB) (136-35-6)	DAAB is used as an intermediate, complexing agent, polymer additive and also to promote adhesion of natural rubber to steel.	Results of NTP Toxicology Report (TOX-73) that demonstrated DAAB is quantitatively metabolized to benzene (a known human carcinogen).
Diethanolamine (DEA) (111-42-2)	DEA is used in the preparation of surfactants used in liquid laundry, dishwashing detergents, cosmetics, shampoos, and hair conditioners. DEA is also used in metal working fluids, in textile processing, industrial gas purification and as an anticorrosion agent.	Results of NTP Bioassay (TR-478, 1999) that reported clear evidence of carcinogenic activity in male and female B6C3F1 mice.
Hepatitis B Virus (HBV)	HBV is a small DNA-enveloped virus that is transmitted by percutaneous or permucosal exposure to infectious blood or body fluids that contain blood.	IARC[1] finding of sufficient evidence of carcinogenicity in humans (Vol. 59, 1994).
Hepatitis C Virus (HCV)	HCV is an RNA-enveloped virus that is transmitted mainly by percutaneous exposure to infectious blood and less efficiently by permucosal exposure to infectious blood or body fluids that contain blood.	IARC[1] finding of sufficient evidence of carcinogenicity in humans (Vol. 59, 1994).
High Risk Human Papillomaviruses (HPVs)	HPVs are small, non-enveloped viruses that infect oral and genital mucosa. HPV infections are common throughout the world.	IARC[1] finding of sufficient evidence of carcinogenicity in humans (Vol. 70, 1997).
X-Radiation and Gamma (γ)-Radiation	The major exposures of concern for cancer from X- and γ-radiation are from the past use of atomic weapons and from medical uses of radiation.	IARC[1] finding of sufficient evidence of carcinogenicity in humans (Vol. 75, 2000).
Neutrons	Exposure to neutrons normally occurs from a mixed irradiation field in which neutrons are a minor component. The exceptions are exposure of patients to neutron radiotherapy beams and exposures of aircraft passengers and crew.	IARC[1] finding of sufficient evidence of carcinogenicity in humans (Vol. 75, 2000).

Nomination (CAS No.)	Primary Uses or Exposures	Basis of Nomination
Occupational Exposure to Lead or Lead Compounds	Major occupational exposures are in the lead smelting and refining industries, battery-manufacturing plants, steel welding or cutting operations, construction, and firing ranges.	Recent published data that indicate an excess of cancers in workers exposed to lead and lead compounds.
Naphthalene (91-20-3)	Naphthalene is used as an intermediate in the synthesis of many industrial chemicals, an ingredient in some moth repellants and toilet bowl deodorants, as an antiseptic for irrigating animal wounds and to control lice on livestock and poultry.	Results of NTP Bioassay (TR-500, 2000) that reported clear evidence of carcinogenicity in male & female rats and some evidence in female mice.
Nitrobenzene (98-95-3)	Nitrobenzene is used mainly in the production of aniline, itself a major chemical intermediate in the production of dyes.	IARC[1] finding of sufficient evidence of carcinogenicity in experimental animals (Vol. 65, 1996).
Nitromethane (75-52-5)	Nitromethane is used in specialized fuels, in explosives and in the synthesis of nitromethane derivatives, pharmaceuticals, agricultural soil fumigants and industrial antimicrobials. In the past it was used as a chemical stabilizer to prevent the decomposition of various halogenated hydrocarbons such as metal degreasers and aerosol propellants.	Results of NTP Bioassay (TR 461, 1997) that reported clear evidence of carcinogenicity in male & female mice and clear evidence in female rats.
4,4’-Thiodianiline (139-65-1)	4,4’-Thiodianiline has been produced commercially since the early 1940’s as an intermediate of several diazo dyes.	IARC[1] finding of sufficient evidence of carcinogenicity in experimental animals (Suppl 7, 1987) and result of NTP Bioassay studies (TR-047, 1978) that demonstrated clear evidence of carcinogenicity in mice and rats.

[1] International Agency for Research on Cancer (IARC)

APPENDIX 1

AGENCY STAFF AND CONTACT INFORMATION

The following listing (alphabetical by agency) includes NTP agency program leaders, project officers, and other key agency staff listed in this plan.

Name	Telephone Number	E-mail Address
NCTR/FDA		
Allaben, William, Dr.	(870) 543-7211	wallaben@nctr.fda.gov
Anson, Jeanne, Ms.	(870) 543-7359	janson@nctr.fda.gov
Beland, Fred, Dr.	(870) 543-7216	fbeland@nctr.fda.gov
Boudreau, Mary, Dr.	(870) 543-7526	mboudreau@nctr.fda.gov
Cerniglia, Carl, Dr.	(870) 543-7341	ccerniglia@nctr.fda.gov
Chou, Ming, Dr.	(870) 543-7661	mchou@nctr.fda.gov
Culp, Sandra, Dr.	(870) 543-7941	sculp@nctr.fda.gov
Delclos, Barry, Dr.	(870) 543-7124	bdelclos@nctr.fda.gov
Ferguson, Sherry, Dr.	(870) 543-7589	sferguson@nctr.fda.gov
Heflich, Robert, Dr.	(870) 543-7493	rheflich@nctr.fda.gov
Howard, Paul, Dr.	(870) 543-7137	phoward@nctr.fda.gov
Jackson, C. Darnell, Dr.	(870) 543-7553	cjackson@nctr.fda.gov
Kadlubar, Fred, Dr.	(870) 543-7204	fkadlubar@nctr.fda.gov
Kodell, Ralph, Dr.	(870) 543-7008	rkodell@nctr.fda.gov
Manjanatha, Mugimane, Dr.	(870) 543-7098	mmanjanatha@nctr.fda.gov
Moore, Martha, Dr.	(870) 543-7050	mmmoore@nctr.fda.gov
Morris, Suzanne, Dr.	(870) 543-7580	smorris@nctr.fda.gov
Reed, Josephine, Ms.	(870) 5437407	mreed@nctr.fda.gov
Scallet, Andy, Dr.	(870) 543-7146	ascallet@nctr.fda.gov
Slikker Jr., William, Dr.	(870) 543-7203	wslikker@nctr.fda.gov
Turesky, Robert, Dr.	(870) 543-7301	rturesky@nctr.fda.gov
Witt, William, Dr.	(870) 543-7949	wwitt@nctr.fda.gov
NIEHS/NIH		
Abdo, Kamal, Dr.	(919) 541-7819	abdok@niehs.nih.gov
Afshari, Cynthia, Dr.	(919) 541-1310	afshari@niehs.nih.gov
Alden, Charles, Dr.	(919) 541-5722	alden@niehs.nih.gov
Baird, Donna, Dr.	(919) 541-2786	baird@niehs.nih.gov
Bell, Doug, Dr.	(919) 541-7686	bell1@niehs.nih.gov
Bernheim, Naomi, Ms.	(919) 541-5085	bernheim@niehs.nih.gov
Bishop, Jack, Dr.	(919) 541-1876	bishop@niehs.nih.gov
Bonner, James, Dr.	(919) 541-0766	bonnerj@niehs.nih.gov
Boorman, Gary, Dr.	(919) 541-4330	boorman@niehs.nih.gov
Bristol, Douglas, Dr.	(919) 541-2756	bristol@niehs.nih.gov
Bucher, John, Dr.	(919) 541-4532	bucher@niehs.nih.gov
Burka, Leo, Dr.	(919) 541-4667	burka@niehs.nih.gov
Burkhart, James, Dr.	(919) 541-3280	burkhart@niehs.nih.gov
Caspary, William, Dr.	(919 541-2150	caspary@niehs.nih.gov
Chan, Po-Cheun, Dr.	(919) 541-7581	chanp@niehs.nih.gov
Chhabra, Rajendra, Dr.	(919) 541-3386	chhabrar@niehs.nih.gov
Chignell, Colin, Dr.	(919) 541-4575	chignell@niehs.nih.gov
Cooper, Glinda, Dr.	(919) 541-0799	cooper1@niehs.nih.gov
Cunningham, Michael, Dr.	(919) 541-3799	cunning1@niehs.nih.gov
Darden, Thomas, Dr.	(919) 541-4933	darden@niehs.nih.gov
Davis, Barbara, Dr.	(919) 541-2764	davis1@niehs.nih.gov

Name	Telephone Number	E-mail Address
Devereux, Theodora, Ms.	(919) 541-3241	devereux@niehs.nih.gov
Dinse, Gregg, Dr.	(919) 541-4931	dinse@niehs.nih.gov
Dixon, Darlene, Dr.	(919) 541-3814	dixon@niehs.nih.gov
Dunnick, June, Dr.	(919) 541-4811	dunnickj@niehs.nih.gov
Dunson, David, Dr.	(191) 541-3033	dunson1@niehs.nih.gov
Eastin, William, Dr.	(919) 541-7941	eastin@niehs.nih.gov
French, John, Dr.	(919) 541-2569	french@niehs.nih.gov
Germolec, Dori, Dr.	(919) 541-3230	germolec@niehs.nih.gov
Ghanayem, Burhan, Dr.	(919) 541-3369	ghanayem@niehs.nih.gov
Goldstein, Joyce, Dr.	(919) 541-4495	goldste1@niehs.nih.gov
Hailey, J. Richard, Dr.	(919) 541-0294	hailey@niehs.nih.gov
Harry, G. Jean, Dr.	(919) 541-0927	harry@niehs.nih.gov
Haseman, Joseph, Dr.	(919) 541-4996	haseman@niehs.nih.gov
Herbert, Ronald, Dr.	(919) 541-4613	herbert@niehs.nih.gov
Hoppin, Jane, Dr.	(919) 541-7622	hoppin1@niehs.nih.gov
Huff, James, Ph.D	(919) 541-3780	huff1@niehs.nih.gov
Irwin, Richard, Dr.	(919) 541-3340	irwin@niehs.nih.gov
Jameson, C. William, Dr.	(919) 541-4096	jameson@niehs.nih.gov
Kamel, Freya, Dr.	(919) 541-1581	kamel@niehs.nih.gov
Kohn, Michael, Dr.	(919) 541-4929	kohn@niehs.nih.gov
Korach, Kenneth, Dr.	(919) 541-3512	korach@niehs.nih.gov
Langenbach, Robert, Dr.	(919) 541-7558	langenb1@niehs.nih.gov
London, Stephanie, Dr.	(919) 541-5772	london2@niehs.nih.gov
Longnecker, Matthew, Dr.	(919) 541-5118	longnec1@niehs.nih.gov
Malling, Heinrich, Dr.	(919) 541-3378	malling@niehs.nih.gov
Maronpot, Robert, Dr.	(919) 541-4861	maronpot@niehs.nih.gov
Mason, Ronald, Dr.	(919) 541-3910	mason4@niehs.nih.gov
Masten, Scott, Dr.	(919) 541-5710	masten@niehs.nih.gov
Melnick, Ronald, Dr.	(919) 541-4142	melnickr@niehs.nih.gov
Merrick, B. Alex, Dr.	(919) 541-1531	merrick@niehs.nih.gov
Miller, David, Dr.	(919) 541-3235	miller@niehs.nih.gov
Moorman, Michael, Mr.	(919) 541-0598	moorma@niehs.nih.gov
Morgan, Dan, Dr.	(919) 541-2264	morgand@niehs.nih.gov
Newbold, Retha, Ms.	(919) 541-0738	newbold1@niehs.nih.gov
Olden, Kenneth, Dr.	(919) 541-3201	olden@niehs.nih.gov
Orzech, Denise, Ms.	(919) 541-5717	orzech@niehs.nih.gov
Portier, Christopher, Dr.	(919) 541-4999	portier@niehs.nih.gov
Pritchard, John, Dr.	(919) 541-4054	pritcha3@niehs.nih.gov
Rao, Ghanta, Dr.	(919) 541-7899	rao@niehs.nih.gov
Rogan, Walter, Dr.	(919) 541-4578	rogan@niehs.nih.gov
Rowley, Michael, Dr.	(919) 541-3436	rowley@niehs.nih.gov
Roycroft, Joseph, Dr.	(919) 541-3627	roycroft@niehs.nih.gov
Sandler, Dale, Dr.	(919) 541-4668	sandler@niehs.nih.gov
Shelby, Michael, Dr.	(919) 541-3455	shelby@niehs.nih.gov
Sills, Robert, Dr.	(919) 541-0180	sills@niehs.nih.gov
Smith, Cynthia, Dr.	(919) 541-3473	smith19@niehs.nih.gov
Soward, Sharon, Ms.	(919) 541-5132	soward@niehs.nih.gov
Stasiewicz, Stanley	(919) 541-7638	stasiew1@niehs.nih.gov
Stokes, William, Dr.	(919) 541-7997	stokes@niehs.nih.gov
Taylor, Jack, Dr.	(919) 541-4631	taylor@niehs.nih.gov
Tennant, Raymond, Dr.	(919) 541-4141	tennant@niehs.nih.gov
Tomer, Kenneth, Dr.	(919) 541-1966	tomer@niehs.nih.gov
Travlos, Greg, Dr.	(919) 541-0653	travlos@niehs.nih.gov
Umbach, David, Dr.	(919) 541-4939	umbach@niehs.nih.gov
Walker, Nigel, Dr.	(919) 541-4893	walker3@niehs.nih.gov
Weinberg, Clarice, Dr.	(919) 541-4927	weinberg@niehs.nih.gov
Wilcox, Allen, Dr.	(919) 541-4660	wilcox@niehs.nih.gov
Wiseman, Roger, Dr.	(919) 541-3225	wiseman@niehs.nih.gov

Name	Telephone Number	E-mail Address
Wolfe, Mary, Dr.	(919) 541-3971	wolfe@niehs.nih.gov
Wright, Larry, Dr.	(919) 541-3426	wright1@niehs.nih.gov
Zeldin, Darryl, Dr.	(919) 541-1169	zeldin@niehs.nih.gov

NIOSH/CDC

Antonini, James, Dr.	(304) 285-6244	jga6@cdc.gov
Biagini, Raymond, Dr.	(513) 533-8196	reb4@cdc.gov
Cheever, Kenneth, Mr.	(513) 533-8193	klc1@cdc.gov
DeBord, D. Gayle Dr.	(513) 533-8212	ded4@cdc.gov
Ding, Min, Dr.	(304) 285-6229	mid5@cdc.gov
Frasch, H. Frederick, Dr.	(304) 285-5755	hbf9@cdc.gov
Frazer, David, Dr.	(304) 285-5872	dgf1@cdc.gov
Hanley, Kevin, Dr.	(513) 841-4113	khanley@cdc.gov
Hubbs, Ann, Dr.	(304) 285-6128	afh0@cdc.gov
Huffman, Linda Dr.	(304) 285-6144	ljh3@cdc.gov
Kesner, James Dr	(513) 533-8208	jsk4@cdc.gov
Kommineni, Choudari, Dr.	(304) 285-6160	cdk2@cdc.gov
Krieg, Edward, Dr.	(513) 533-8160	erk3@cdc.gov
Lewis, Daniel, Dr	(304) 285-5720	dml1@cdc.gov
Lindsley, William, Dr.	(304) 285-6336	wdl7@cdc.gov
Lotz, W. Gregory Dr.	(513) 533-8153	wlotz@cdc.gov
Luster, Michael, Dr.	(304) 285-5940	mluster@cdc.gov
Lynch, Dennis, Dr.	(513) 533-8213	dlynch@cdc.gov
Ma, Jane, Dr.	(304) 285-5844	jym1@cdc.gov
Meade, B. Jean Dr.	(304) 285-5809	bhm8@cdc.gov
Mercer, R., Dr.	(304) 285-6157	rpm7@cdc.gov
Moorman, William, Mr.	(513) 533-8275	wmoorman@cdc.gov
Munson, Albert E. Dr.	(304) 285-6121	akm5@cdc.gov
Murono, Eisuke, Dr.	(304) 285-6145	eem8@cdc.gov
Ong, T-M, Dr.	(304) 285-5817	too2@cdc.gov
Pringle, Leon, Dr.	(304) 285-6274	lgp0@cdc.gov
Reynolds, Jeff, Dr.	(304) 285-6238	jsr0@cdc.gov
Ruder, Avima Dr.	(513) 841-4440	amr2@cdc.gov
Sanderson, Wayne, Dr.	(513) 841-4476	wts1@cdc.gov
Savage, Russell, Dr.	(513) 533-8289	ras6@cdc.gov
Schrader, Steven, Dr.	(513) 533-8210	sms4@cdc.gov
Schulte, Paul, Dr.	(513) 533-8302	pas4@cdc.gov
Shi, Xianglin, Dr.	(304) 285-6158	xshi@cdc.gov
Siegel, Paul, Dr.	(304) 285-5855	pds3@cdc.gov
Shvedova, Anna, Dr.	(304) 285-6177	ats1@cdc.gov
Snawder, John, Dr.	(513) 533-8496	jts5@cdc.gov
Soderholm, Sidney, Dr.	(304) 285-6034	sgs2@cdc.gov
Streicher, Robert, Dr.	(513) 841-4296	rstreicher@cdc.gov
Wang, Jin, Dr.	(304) 285-6329	juw9@cdc.gov
Waters, Thomas, Dr.	(513) 533-8147	trw1@cdc.gov
Striley, Cynthia, Dr.	(513) 533-8123	cstriley@cdc.gov
Tinkle, Sally, Dr.	(304) 285-5841	sft3@cdc.gov
Toraason, Mark, Dr.	(513) 533-8207	mtoraason@cdc.gov
Wallace, William, Dr.	(304) 285-6096	wew2@cdc.gov
Weisman, David, Dr.	(304) 285-6261	dweissman@cdc.gov
Weston, Ainsley, Dr.	(304) 285-6221	agw8@cdc.gov
Whelan, Elizabeth, Dr.	(513) 841-4437	ewhelan@cdc.gov

APPENDIX 2

NTP BOARD OF SCIENTIFIC COUNSELORS

Name and Title	Affiliation	Term Ends	Board Service[1]	Expertise
George Bailey, Jr., Ph.D. Professor of Food Toxicology and Director, Marine/Freshwater Biomedical Sciences Center	Oregon State University Corvallis, OR	12/27/02	Board	Alternative Methods, Xenobiotic Metabolism
Aaron E. Blair, Ph.D., M.P.H. Chief, Occupational Epidemiology Branch, EBP, DCEG	National Cancer Institute, National Institute of Health Bethesda, MD	6/30/05	RoC	Molecular and Cancer Epidemiology, Occupational Health
Kim Boekelheide, M.D., Ph.D. Professor, Division of Biology and Medicine Department of Pathology and Laboratory Medicine	Brown University Providence, RI	6/30/04	Board	Reproductive Toxicology, Pathology, Molecular Biology
George Bonney, Ph.D. Professor, Microbiology Director Statistical Genetics and Bioinformatics Unit, National Human Genome Center	Howard University Washington, DC	6/30/04	Board RoC	Genetic Epidemiology, Carcinogenesis, Biostatistics
Hillary M. Carpenter, III, Ph.D. Toxicologist	Minnesota Department of Health St. Paul, MN	6/30/04	Board RoC	Toxicology, Risk Assessment, Public Health
Gail Charnley, Ph.D. Principal	Health Risk Strategies Washington, DC	6/30/05	RoC	Environmental Health Risk Assessment, Risk Management, Public Policy, Toxicology
Harvey Checkoway, Ph.D., M.P.H. Professor, Department of Environmental Health and Epidemiology School of Public Health and Community Medicine	University of Washington Seattle, WA	6/30/05	Board	Environmental and Occupational Epidemiology, Exposure Assessment, Molecular Epidemiology
Samuel M. Cohen, M.D., Ph.D. Professor and Chairman Department of Pathology and Microbiology	University of Nebraska Medical Center Omaha, NE	6/30/04	Board	Chemical Carcinogenesis, Pathology, Molecular Biology, Environmental Toxicology
Norman R. Drinkwater, Ph.D. Director, McArdle Laboratory for Cancer Research	University of Wisconsin-Madison Madison, WI	12/27/02	Board TRRS	Experimental Carcinogenesis
Michael R. Elwell, D.V.M., Ph.D. Research Advisor Pathology, Drug Safety Evaluation	Pfizer Global Research and Development Groton, CT	6/30/05	TRRS	Pathology, Environmental Toxicology, Alternative Animal Models, Carcinogenesis
Thomas A. Gasiewicz, Ph.D. Professor, Department of Environmental Medicine Environmental Health Sciences Center	University of Rochester School of Medicine Rochester, NY	6/30/05	Board	Immunotoxicology, Biochemistry, Molecular Biology
John R. Froines, Ph.D. Professor and Director UCLA Center for Occupational and Environmental Health	UCLA School of Public Health Los Angeles, CA	6/30/03	RoC	Occupational Health
Howard Frumkin, M.D., Dr.P.H. Professor, Dept of Environmental and Occupational Health	The Rollins School of Public Health, Emory University Atlanta, GA	6/30/05	RoC	Occupational Health, Environmental and Clinical Epidemiology, Internal Medicine
Lynn Goldman, M.D.	Johns Hopkins University	12/27/02	Board	Pediatrics, Health

Name and Title	Affiliation	Term Ends	Board Service[1]	Expertise
Professor, Department of Environmental Health Sciences	School of Public Health Baltimore, MD			Regulation
Irva Hertz-Picciotto, Ph.D., M.P.H. Department of Epidemiology and Preventive Medicine	University of California-Davis Davis, CA	6/30/04	RoC	Biostatistics, Epidemiologic Methods, Risk Assessment, Occupational and Environmental Epidemiology
Shuk-Mei Ho, Ph.D. Professor of Surgery and Cell Biology Department of Surgery, Division of Urology	University of Massachusetts Medical School Worcester, MA	6/30/05	TRRS	Reproductive Biology, Molecular and Cellular Biology, Endocrinology, Carcinogenesis
Margaret R. Karagas, Ph.D. Associate Professor, Section of Biostatistics/Epidemiology	Department of Community and Family Medicine Dartmouth Medical School Labanon, NH	6/30/04	RoC	Epidemiologic Methods, Biostatistics, Molecular, Cancer and Environmental Epidemiology
James E. Klaunig, Ph.D. Director, Division of Toxicology Professor, Department of Pharmacology and Toxicology	Indiana University School of Medicine Indianapolis, IN	6/30/03	TRRS	Toxicology, Experimental Carcinogenesis, Public Health
Grace K. Lemasters, Ph.D. Professor and Director Division of Epidemiology and Biostatistics, Department of Environmental Health	University of Cincinnati, College of Medicine Cincinnati, OH	12/27/02	Board	Occupational, Reproductive Epidemiology
David E. Malarkey, DVM, Ph.D. Assistant Professor of Pathology, Department of Microbiology, Pathology and Parasitology	College of Veterinary Medicine North Carolina State University Raleigh, NC	6/30/04	TRRS	Pathology, Lab Animal Medicine
Donald R. Mattison, M.D.	National Institute of Child Health and Human Development	6/30/03	Board	Reproductive and Developmental Risk Assessment
Rafael Moure-Eraso, Ph.D, C.I.H. Professor, Department of Work Environment	University of Massachusetts Lowell, College of Engineering Lowell, MA	12/27/02	Board RoC	Industrial Hygiene Worker Health
Barbara C. Pence, Ph.D. Professor, Department of Pathology	Texas Tech University, Health Sciences Center Lubbock, TX	6/30/05	RoC	Microbiology, Comparative Pathology, Experimental Carcinogenesis
Walter W. Piegorsch, Ph.D. Professor of Statistics Director of Undergraduate Studies Department of Statistics	University of South Carolina Columbia, SC	6/30/04	RoC TRRS	Biostatistics, Risk Assessment
James A. Popp, D.V.M., Ph.D. Vice President, Nonclinical Drug Safety Evaluation & Pharmacokinetics/Drug Metabolism	Purdue Pharma, L.P. Ardsley, NY	6/30/05	RoC	Pathology, Carcinogenesis, Animal Models
Stephen M. Roberts, Ph.D. Professor, Department of Physiological Sciences	College of Veterinary Medicine University of Florida Gainesville, FL	6/30/05	TRRS	Toxicology, Pharmacology, Cell Biology, Animal Models
Allan H. Smith, M.D., Ph.D. Professor of Epidemiology	University of California, Berkeley School of Public Health Berkeley, CA	6/30/03	RoC	Epidemiology
Richard D. Storer, Ph.D., M.P.H. Senior Investigator, Department of Genetic and Cellular Toxicology	Merck Research Laboratories West Point, PA	6/30/04	TRRS	Environmental and Genetic Toxicology, Molecular Carcinogenesis, Alternative Models

Name and Title	Affiliation	Term Ends	Board Service[1]	Expertise
Mary Anna Thrall, DVM Professor, Department of Pathology	Colorado State University College of Veterinary Medicine & Biomedical Sciences Fort Collins, CO	6/30/04	TRRS	Clinical Pathology, Lab Animal Medicine
Mary Vore, Ph.D. Director, Graduate Center for Toxicology	University of Kentucky Lexington, KY	6/30/05	TRRS	Toxicology, Pharmacology, Cell Biology
Cheryl Lyn Walker, Ph.D. Professor of Carcinogenesis, Department of Carcinogenesis	The University of Texas M.D. Anderson Cancer Center Smithville, TX	6/30/05	Board	Molecular Carcinogenesis, Alternative Animal Models, Reproductive Biology
Bruce S. Weir, Ph.D. William Neal Reynolds Professor of Statistics and Genetics	North Carolina State University Department of Statistics Raleigh, NC	6/30/05	Board	Biostatistics, Bioinformatics, Quantitative Genetics
Expert Consultant				
David H. Phillips, Ph.D., DSc, FRCPath Research Scientist, Institute of Cancer Research	Haddow Laboratories Sutton, England	6/30/03	RoC TRRS	Molecular Epidemiology, Carcinogenesis

[1]Board Service
Board = Serves as member on the parent Board
RoC = Serves on the NTP Board of Scientific Counselors Report on Carcinogens Subcommittee
TRRS = Serves on the NTP Board of Scientific Counselors Technical Reports Review Subcommittee